The Role of Weather and Topography in the Development of *Dothistroma septosporum*.

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BSc, University of Northern British Columbia, 2005

Thesis Submitted In Partial Fulfillment Of

The Requirements For The Degree Of

Master Of Science

In

Natural Resources And Environmental Studies

(Biology)

University of Northern British Columbia

April 2009

Your file Votre référence
ISBN: 978-0-494-48793-8
Our file Notre référence
ISBN: 978-0-494-48793-8

Abstract

Dothistroma septosporum (Dorog.) Morelet is recognized worldwide as a foliar disease of pine trees and was responsible for a severe outbreak in northwestern British Columbia. Plots equipped with weather sensors were established in lodgepole pine plantations for weekly monitoring, and aerial survey data was used to investigate topographic effects. Symptoms were observed from early June to late September. Conidia were detected late June to late August. Mixed effects models identified temperature thresholds lower than lab-based predictions, and indicated that leaf wetness plays a greater role than relative humidity in symptom development. The telomorph was rarely observed in this study, suggesting that sexual reproduction is rare in northwestern BC. Disease severity decreased with increasing elevation and south-facing aspects. Slope and proximity to water bodies had no influence on disease severity. When sufficient inoculum levels are present, low-lying areas where moist air can be retained for long periods facilitate *D. septosporum* development.

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ACKNOWLEDGEMENTS

The support and advice of a wide variety of faculty, colleagues, and friends helped me to complete this project. First and foremost I would like to thank my supervisor, Kathy Lewis, for the opportunity to lead this project. I would also like to thank Brian Aukema for all his patience with my burgeoning statistical ability, and Alex Woods for his encouragement and technical support.

The fantastic community of support within UNBC helped this project progress to completion. My numerous requests were met by staff in the Dendrochronology Lab, including Doug Thompson, John Orlowsky, Steve Storch, Chris Konchalski, and Dean Labonte. Many questions were also answered by Roger Wheate and the GIS and Remote Sensing Lab.

Tremendous support also came from the staff at the BC Ministry of Forests and Range branch offices in Hazleton and Smithers through Kate Pottinger and Erin Havard. Support was also found in the field and by email from Paul Hanna and Dieter Marder at Silverwood Consulting. I am also very thankful for the hospitality of the Kispiox and Kitwanga valley communities.

Finally, I would like to especially thank Chris Konchalski for his patient hard work and positive attitude throughout the project. Many thanks to Dylan Richards, Kamalprit Chohan, Krystan Braun, and numerous others not mentioned here for their incredible behind-the-scenes support.

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Dothistroma septosporum (Dorog.) Morelet, causal agent of red band needle blight, also known as Dothistroma needle blight, is a foliar fungus that infects and kills the needles of pine trees. This fungal pathogen is characterized by its red banding appearance in areas along needles where infection has been successful. The mycotoxin dothistromin is responsible for the red color of the bands (Bradshaw 2004). Infection results in the death of the needle and defoliation of the tree.

Dothistroma needle blight is recognized worldwide, affecting over 60 *Pinus* species in 45 countries (Ivory 1994). It is responsible for extensive damage in exotic plantations in the southern hemisphere in countries such as Chile, Kenya, Australia, and New Zealand (Ivory 1967; Gibson 1972). Reports from the USA, UK, Czech Republic, Austria, Serbia, Ukraine, and the European part of south-western Russia suggest that Dothistroma needle blight is on the rise in the northern hemisphere (Karadžić 1994; Taylor and Walla 1999; Brown *et al.* 2003; Bednarova *et al.* 2007; Barnes *et al.* 2008). In the northern hemisphere it is thought to be native to pines (Harrington and Wingfield 1998), and until recently was not considered an important pest.

Found throughout British Columbia (BC), the first recorded incidence of Dothistroma needle blight (herein referred to as Dothistroma) occurred in the early 1960s when it was noted on three native and six exotic pine species (Parker and Collis 1966). Trees less than 10-years old appeared to be the most susceptible (Parker and Collis 1966). The next record occurred in 1984-1986, when Dothistroma was observed in northwestern BC (FIDs 1984, 1985, 1986). Little attention had been given to Dothistroma as it has not been responsible for

any large outbreaks until recently. In the past, natural controls such as low summer minimum temperatures and precipitation levels were thought to have kept disease levels low (Welsh 2007).

In recent years a severe outbreak of Dothistroma has been developing in the Skeena Stikine and Kalum Forest Districts in northwestern BC. Dothistroma was found to be the most prevalent pest in a survey of 100 randomly selected lodgepole pine (*Pinus contorta* var *latifolia* Dougl. Ex Loud.) plantations (Woods 2003). Further investigation revealed that 94% of lodgepole pine plantations in these forest districts suffered some degree of infection, and damage in these plantations ranged from low levels of infection to nearly 100% mortality (Woods 2003; Woods *et al.* 2005).

Lodgepole pine, along with spruce, is an important species in the interior of the province, comprising 14.9 million hectares in BC (Province of British Columbia 2001; Pedersen 2004). Given its wide range of ecological tolerance, lodgepole pine is often the only tree species that will grow on infertile soils and is one of the most commonly used species in reforestation because it also grows quickly (Lotan and Critchfield 1990). In light of the mountain pine beetle epidemic that is predicted to kill 80% of susceptible lodgepole pine in the province's interior by the end of the current outbreak (McGarrity and Hoberg 2005), the economic impacts of further lodgepole pine mortality in northwestern BC could be severe.

Temperature and needle wetness are key weather variables in the development of *D. septosporum* (Bulman 1993). Studies from New Zealand have found that infection and development of symptoms can occur between 5°C and 26°C, with infection at lower temperatures thought to be dependent on extended periods of humidity (Gilmour and Crockett 1972, cited in Bradshaw 2004). Studies based on radiata pine (*Pinus radiata* D.

Don, also known as Monterey pine) found that stromata develop sooner under conditions of higher temperature and longer wetness periods (Gadgil 1974). Weather conditions triggering *D. septosporum* symptom development in lodgepole pine plantations are not well known. Warm, wet summers and cool, wet falls are thought to be contributing climatic factors to the severity of the current outbreak (Woods *et al.* 2003). However temperature, relative humidity, and leaf wetness thresholds for *D. septosporum* infection and reproduction in lodgepole pine are yet to be identified. The purpose of this research is to identify site and weather conditions that contribute to the development of *D. septosporum* in northwestern BC.

1.2 The pathogen

The origins of *D. septosporum* are conflicting in the literature, with suggestions ranging from the Himalayas to high altitude rainforests in South America (Evans 1984; Ivory 1994). In northwestern BC *D. septosporum* is thought to be endemic, with outbreaks occurring periodically for the last 174 years (Welsh 2007). Previously Dothistroma has been classified into three varieties according to mean conidial length: *Dothistroma pini* Hulbary var. *pini*, *Dothistroma pini* Hulbary var. *linearis* Thyr and Shaw, and *Dothistroma pini* Hulbary var. *keniensis* (Ivory 1967). More recently this classification has been dismissed on the basis of genetic investigation (Barnes *et al.* 2004). Barnes *et al.* (2004) proposed two species of Dothistroma: *Dothistroma septosporum* (found worldwide) and *Dothistroma pini* (found in north central USA, Ukraine, and southwestern Russia). So far DNA sequence comparisons are the most reliable way to conclusively distinguish between the two Dothistroma species (Barnes *et al.* 2008). Until recently *D. pini* had only been found in north central USA (Barnes *et al.* 2004), but its presence on native pine in Ukraine and

southwestern Russia has raised questions as to whether *D. pini* could have originated from Europe and was accidentally introduced into the USA (Barnes *et al.* 2008).

D. septosporum is the anamorph, or asexual stage, of *Mycosphaerella pini* E. Rostrup (see Figure 1.1). The disease cycle of *M. pini* is completed in one year on the lower mainland and coast of BC, but requires two years in most other parts of North America (Hunt 1981). *D. septosporum* is the most commonly observed reproductive stage and asexual spores, or conidia, are the main source of inoculum (Hunt 1981; Bradshaw 2004). They are usually dispersed by rainsplash, though there is some evidence of conidial dispersal by wind and cloud transport (Bradshaw 2004). Gibson *et al.* (1964) found that the majority of conidia were released during periods of rain or heavy mist when infected foliage was dripping wet. No conidia were released when a strong current of air was directed at dry or moist foliage (Gibson *et al.* 1964). These conidia retained viability for a maximum of 4-6 months, even on abscised needles (Gadgil 1970). Infected first- and second-year needles remained attached to the tree over the winter, and were the main sources of inoculum when spores were released the following spring and summer (Brown *et al.* 2003). High inoculum levels may be necessary for pathogen success as even under favourable conditions very large numbers of spores were needed to achieve moderate levels of infection (Bulman *et al.* 2004).

Wet spores can germinate and penetrate a needle even in dry conditions, but symptom formation and development require high humidity (Gadgil 1977). Infection and development of symptoms can occur between 5°C and 26°C, with infection at lower temperatures thought to be dependent on extended periods of humidity (Gilmour and Crockett 1972, cited in Bradshaw 2004). A period of 10 hours or more of needle wetness is usually required for successful infection, and long dry periods after infection generally lead to less severity and

slow development of stromata (Bulman 1993; Bradshaw 2004). One or more germ tubes emerge from a germinating spore, though whether their direction of growth is targeted or random is conflicting in the literature (Peterson 1966, cited in Gadgil 1967; Gadgil 1967; Muir and Cobb 2005). Germ tube growth may follow water gradients from spores to stomata (Peterson and Walla 1978). A more recently published study in North America found that germ tubes grow directly towards and enter the nearest epistomatal opening, and germ tubes enter more often on abaxial than adaxial surfaces of the needle (Muir and Cobb 2005). At the entry site the germ tube undergoes a morphological change, forming an appressorium that adheres to the leaf surface and facilitates penetration (Peterson and Walla 1978). Penetration may terminate in substomatal vesicles that form just below the guard cells, which enable the pathogen to persist in needles until conditions are suitable for colonization (Muir and Cobb 2005). Hyphae then grow into intra- and intercellular regions of the mesophyll layer of the needle tissue, and after 32-114 days host cells collapse (Ivory 1972; Bradshaw 2004).

In BC infection of needles of all ages occurs throughout the year on the coast and from spring to autumn in the interior (Hunt 1981). Stromata, the black fruiting bodies of the anamorph, mature in the needle tissue and lesions are formed, bearing 1-12 stromata usually covered by small epidermal flaps (Phillips and Burdekin 1982). Although stromata may begin to develop in the fall, most mature in the spring following the year of infection (Peterson 1969). They emerge from early spring to late summer, and mature throughout the growing season (Peterson 1969). The onset of symptom development consists of short-lived deep-green bands, followed by the appearance of yellow and tan spots on needles (Peterson 1982; Coops *et al.* 2003). The bands, or lesions, then turn bright red; hence the name 'red

band needle blight.' Needle tips distal to lesions turn brown and die, while the base of the needles generally remain green (Hunt 1981).

Within a lesion hyphal growth is restricted to necrotic tissue, and extension of necrosis beyond the infected area indicates that host cells are killed by a toxin or host defense response (Gadgil 1967; Ivory 1972; Bradshaw 2004). Adjacent to the red lesions are yellow areas of necrotic tissue, sometimes flanked by areas of dark green tissue containing highly lignified cells (Franich *et al.* 1986). The change from green to red can be sharp or interposed with yellowish areas between green area and red band (Gadgil 1967).

The red colour of the lesions visible on an infected needle is due to the presence of dothistromin, one of 300 mycotoxins that are known mutagens and animal carcinogens (Shain and Franich 1981; Ames *et al.* 1987). Though toxic to human cells, dothistromin is not considered a health hazard to forest workers (Elliot *et al.* 1989; Stoessl *et al.* 1990). Until recently the toxin was thought to be essential for pathogenicity (Schwelm *et al.* 2009).

Infection severity has been found to increase with increasing light intensity, suggesting there is an interaction between dothistromin and photosynthetically-active tissue (Gadgil and Holden 1976; Shain and Franich 1981). Dothistromin is broken down to oxalic acid and CO_2 more efficiently in light (80% breakdown) compared to dark (5-10% breakdown) (Franich *et al.* 1986). This breakdown may be achieved through two ways as suggested by Franich *et al.* (1986): (1) through metabolism by dying cells, possibly by peroxidase-catalyzed oxidation using O_2 or H_2O_2 as an oxidant; or (2) photolytic degradation in the presence of O_2 or H_2O_2, with oxidation stopping once needle tissue has become brown and opaque, preventing the transmission of light. Given that most of the toxin is destroyed in

24 h, Franich *et al.* (1986) suggests that most of the needle damage must be due to the plant's defense response rather than direct toxicity.

While host defense responses are unclear, it is possible that the toxin is not necessary for symptom development. In northwestern BC, Dothistroma needle blight with absence of red banding has been observed in the current outbreak (Alex Woods[1], personal communication, January 16, 2007). Though variation in dothistromin production among strains is observed (Bradshaw *et al.* 2000), its role as a pathogenicity factor requires further investigation (Schwelm *et al.* 2009).

1.2.1 Role of the reproductive stages

The teleomorph of Dothistroma has been reported in Canada, USA, Serbia, Germany, Poland, Yugoslavia, and Portugal (Karadžić 1994; Bradshaw 2004). Generally the sexual stage of fungi that cause needle disease develops towards the end of the growing season and is relatively uncommon (Harrington and Wingfield 1998). This is evident in studies of Dothistroma such as by Funk (1979) who observed the sexual stage of Dothistroma 4-5% of the time when monitoring naturally infected lodgepole pine needles in both dry and wet conditions. In Nebraska USA, Peterson (1973) monitored Dothistroma development and spore production and reported having never observed the sexual stage. Although both reproductive stages can infect needles of all ages (Funk 1985), some literature suggests that the teleomorph is saprophytic as its ascostromata usually form on 2- and 3-year-old needles and only when the needle is completely necrotic (Butin and Richter 1983, cited in Bradshaw 2004; Karadžić 1994).

[1] Alex Woods, Regional Forest Pathologist, Northern Forest Region, 3333 Tatlow Road, Smithers, BC, V0J 2N0, (250) 847-6382.

Latent sexual reproduction in Dothistroma may be incited by different environmental conditions than the asexual stage. In Serbia, Karadžić (1989) observed conidia dispersal from April to October, whereas ascospores dispersed from late June until September. Following this observation, Karadžić (1989) suggested that the importance of conidia is much greater than ascospores because conidia are dispersed over a longer period, and that the fungus is primarily dispersed by the conidia. Further evidence is suggested in Gilmour (1981), whose field studies conflicted with results from Parker (1972, cited in Gilmour 1981). Parker (1972) found that low temperature and high humidity enhanced infection levels, whereas Gilmour (1981) found that infection was favoured by higher temperatures. Gilmour (1981) suggested that the isolate (from ascospores on fruiting bodies on *P. contorta*) used by Parker (1972) may have different temperature requirements than the isolate (from conidia on stromata of *P. radiata*) used by Gilmour (1981).

Genetic recombination facilitated by sexual reproduction may currently be giving rise to more virulent strains of the pathogen. Thus, its appearance in high frequencies may help explain the severity of the current outbreak in northwestern BC where the fungus is thought to be endemic and historically was not problematic. Both the anamorph and teleomorph have been found within the *D. septosporum* population in northwestern BC (Groenewald *et al.* 2007). High levels of genetic variability have been observed in the current outbreak, indicating that sexual reproduction in northwestern BC is common (Dale 2008). Dale (2008) found high genetic diversity within a stand and has suggested that the splash dispersed conidia are not the main method of dispersal within a stand. This study has also provided evidence for the pathogen's ability to disperse over long distances.

Though well documented for the anamorph, environmental conditions triggering the development of the teleomorph have not been outlined in previous literature. Monitoring symptomatic needles for the teleomorph will both ascertain the influence of environmental variables on its development as well as shed light on its contributing role in the current outbreak.

1.3 The host

Dothistroma needle blight is considered one of the most important diseases of pines in the world, and may be the most damaging and prevalent where host species are planted outside of their natural range (Brown *et al.* 2003; Barnes *et al.* 2004). It appears to be endemic on pine in parts of Europe and North America (Gibson 1974). It is currently known to infect over 60 *Pinus* species in 45 countries, and is especially harmful to radiata pine, lodgepole pine, ponderosa pine (*P. ponderosa* Dougl. ex Laws.), Jeffrey pine (*P. jeffreyi* Grev. & Balf.), and European black pine (*P. nigra* Arnold) (Ivory 1994; Karadžić 1994). When conditions are favourable, spread of the disease can be rapid. When Dothistroma was first detected in a radiata pine plantation in October 1965, symptoms were obvious on 6-12 saplings (Cobb and Miller 1968). In April 1966 the fungus was found on more than 100 trees in the plantation (Cobb and Miller 1968).

Trees infected with *D. septosporum* can be defoliated within weeks, and mortality can result from repeated attacks (NRC 2003). New needles are generally resistant, but tend to succumb by the end of the season. In ponderosa and European black pine it has been observed that lesions on second-year needles may be as numerous near the tip as the base, whereas on first-year needles there may be a higher frequency of lesions near the tip (Peterson 1969). This may result from the chemical properties of developing tissues, or the

longer exposure of the distal end (Peterson 1969). The disease typically starts at the base of the lower crown and moves up (Karadžić 1989). The lower crown is usually the most severely affected, and infected trees tend to have thin crowns with discoloured and dead needles (Hunt 1981). Older diseased needles are shed, often forming "lion's-tail" branches, with only terminal needles remaining (Hunt 1981).

The most serious impact of Dothistroma has been a reduction of growth due to defoliation (Bradshaw 2004). Mortality is normally considered rare. A study on radiata pine found surprisingly few effects of severe Dothistroma infection on wood properties. The effects were limited to some loss of diameter growth, which is more marked in the upper than in the lower stem, and a trend towards increasing wood density as growth rates decrease (Harris and McConchie 1978). The impact is not considered significant until defoliation exceeds 25% in the current year's foliage on 50% of the total number of trees in the stand (Whyte 1969, cited in Gibson 1974). Van der Pas (1981) suggests that a *D. septosporum* infection level of 50% diseased needles over several seasons would result in 50% reduction in volume increment. Defoliation by Dothistroma can also affect the form of a tree, reducing growth at the base of the stem and less stem taper overall (Van der Pas 1981).

Resistance to Dothistroma takes two forms: some species remain equally susceptible at all ages, while others become increasingly resistant with age (Gibson 1972). Species of *Pinus* such as knobcone (*P. attenuata* Lemmon), European black, and ponderosa pine do not develop resistance to Dothistroma infection with age (Gadgil 1970). Previously lodgepole pine was thought to develop Dothistroma resistance with age, as radiata pine does (Gibson 1972). However, mature lodgepole pine dying from Dothistroma infection have been observed by Woods *et al.* (2005). Some tree-to-tree variation in susceptibility to *D.*

septosporum can usually be seen, but generally infection levels are fairly evenly distributed within a stand unless the terrain differs significantly (Bulman *et al.* 2004).

Control of Dothistroma has been achieved through fungicide spraying and breeding resistant planting stock (Bradshaw 2004). In the southern hemisphere the spraying of copper fungicide to inhibit the germination of spores is used to manage the disease, and a single spray application can be effective, depending on disease severity and appropriate timing (Gilmour and Noorderhaven 1971; Van der Pas *et al.* 1984; Franich 1988). In the US, application of the fungicidal Bordeaux mixture, a mixture of copper sulfate and hydrated lime, is recommended (Peterson 1982; Agrios 1997). Whether the associated costs of spraying exceed the value of the recovered volume of timber is debatable (Van der Pas *et al.* 1984; Alzamora *et al.* 2004), and it has been suggested that the utilization of resistant breeds of pine would be the best option in order to maximize gains (Dick 1989). Until resistant cultivars can be developed as a more economical control measure, fungicides are among the most economic means of dealing with stem and foliage diseases (Gibson 1974).

The development of resistance stocks has shown promise. In some hosts, such as radiata pine, resistance has been shown to be highly heritable. Ades and Simpson (1990) compared fifty-two clones in twenty-two full-sib families of radiata pine, and calculated that if the best 10% of the clones were selected, infection could have been further reduced to 12%. This is consistent with other studies, in which the development of a breeding stock of radiata pine has reduced mean infection levels by 11-12% (Carson 1989).

1.3.1 Hosts in BC

Dothistroma has been reported on lodgepole and ponderosa pine, both native species to BC (Hunt 1981). It has also been reported on non-native species such as Jeffrey, radiata,

bishop (*Pinus muricata* D. Don), and maritime (*Pinus pinaster* Aiton) pine, as well as some hybrid pine species (NRC 2003). It was previously considered most damaging in plantations of exotic pines, and not generally capable of mortality in mature native stands of lodgepole pine (Peterson 1981, cited in Taylor and Schwandt 1998). Intensive planting of lodgepole pine in the area of the current outbreak appears to have exacerbated disease problems (Woods 2003). According to tree ring analysis, Dothistroma outbreaks have occurred on lodgepole pine since the early 1800s in northwestern BC, although the current outbreak has been the most severe (Welsh 2007).

Lodgepole pine accounts for 27% of the total harvest and 41% of total planting in BC (Province of British Columbia 2007). It has a wide ecological amplitude (Lotan and Critchfield 1990), and in the interior it can be found between 490 m and 3660 m. Because lodgepole pine has little taper and thin bark, it produces higher wood volume for a given diameter and height than many of its associates (Lotan and Critchfield 1990). Its defenses to foliar fungi are preformed; stomatal closure, guard cell size, and waxy cuticles constitute mechanical and physical barriers to infection. Though other mechanisms behind resistance are not well understood, Muir and Cobb (2005) suggest that in some *Pinus* species, an unidentified form of host resistance can be seen after *D. septosporum* has penetrated stomata as substomatal vesicles. Substomatal vesicles are a type of infection structure that form just below the guard cells. *D. septosporum* in radiata pine occasionally formed substomatal vesicles and produced more disease lesions than in bishop pine, in which substomatal vesicles were common and often disintegrated (Muir and Cobb 2005).

Hypersensitive response, in which programmed cell death occurs as a response to infection, may also explain symptom development. It is an induced response by the tree to

produce physical barriers to fungal growth, usually in the form of lignin deposition around invading hyphae (Oliver and Schweizer 1999). This reaction by resistant plants causes cells to lose turgor, turn brown and die, while susceptible cells last considerably longer (Agrios 1988). Franich *et al.* (1986) observed highly lignified cells flanking necrotic tissues killed by *D. septosporum* in radiata pine. If a hypersensitive response occurs in lodgepole pine, then some of the variation seen in Dothistroma symptom development may be due to individual tree resistance in addition to effects of microclimate. However, research investigating this possibility is currently lacking.

In northwestern BC a survey by Woods (2003) found that the severity of infected crowns of lodgepole pine 20 years and younger ranged from less than 5 to more than 90%. Within a 55-year-old stand more than 20% mortality was observed, with 60% of remaining trees having less than 5% full needle compliment (Woods 2003). Woods (2003) believed that the high levels of foliar disease in lodgepole pine plantations resulted from two factors: (1) there had been an increase in concentration of young susceptible hosts on the landscape because of widespread planting of lodgepole pine; and (2) the climate of interior cedar-hemlock zone which consisted of warm, moist summers and cool, wet falls, had become more favourable with even more summer precipitation that is conducive to disease development. Woods *et al.* (2005) went further, suggesting the more severe epidemic may be related to climate change.

1.3.2 Other defoliators

Other fungal defoliators of lodgepole pine in BC include *Lophodermium pinastri* (Shrad.:Fr.) Chev., *Lophodermium seditiosum* Minter, Staley, & Millar, *Davisomycella ampla* (J. J. Davis) Darker, *Hendersonia pinicola* (Wehm.), *Elytroderma deformans* (Weir)

Darker, *Lophodermella concolor* (Dearn.) Darker, and *Mycosphaerella dearnessii* M.E. Barr (Hunt 1981; EMPPO 2005). *L. pinastri*, *L. seditiosum*, *H. pinicola*, and *D. ampla* produce oval black fruiting bodies on dead needles, and only *D. ampla* fruiting bodies are accompanied by an orange-brown band (Hunt 1981). *E. deformans* ascomata appear as narrow black lines on browned needles (Hunt 1981). *L. concolor* apothecia appear as shallow oval depressions that are concolorous with the needle surface (Hunt 1981). None of these foliar diseases produce the red bands as found with Dothistroma needle blight, and none of their fruiting bodies erupt with the distinguishing epidermal flap. *M. dearnessii* (the causal agent of brown spot needle blight) is the foliar disease most likely to be mistaken as Dothistroma needle blight (EMPPO 2005). It is best known for damage caused to longleaf pine (*Pinus palustris* Miller) plantations in southern USA and has been noted in Canada (Harrington and Wingfield 1998). *M. dearnessii* (= *Lecanosticta acicola* (Thümen) H. Sydow) produces brown bands and stromata that erupt similarly to *D. septosporum*, and can only be distinguished by microscopic examination when mature conidia are produced (EMPPO 2005).

1.4 Environment

In addition to a virulent pathogen and susceptible host, suitable environmental conditions are required for a disease outbreak (Agrios 1997). Environmental conditions can affect growth and resistance of the host plant, rate of growth of pathogen, degree of virulence of pathogen, and dispersal by wind, water, vectors, and other disease-development factors (Agrios 1997). In the case of Dothistroma, climatic and topographic conditions may have a significant influence on disease severity (Marks and Hepworth 1986; Woods *et al.* 2005).

1.4.1 Climate

Two of the most important factors in the development of plant disease epidemics are temperature and moisture (Agrios 1997). *D. septosporum* is no exception as it depends on temperature, the duration of the needle-wetness period, and the amount of spores present (Bulman 1993). Wet spores can germinate and penetrate a needle even in dry conditions, but symptom formation and development require high humidity (Gadgil 1977). Generally long dry periods after infection lead to less disease severity and slow the development of stromata (Gadgil 1977; Bradshaw 2004). Under conditions of continuous moisture the incidence of infection is greatly increased, and stromata develop sooner with higher temperatures and longer wetness periods (Gadgil 1974). A field study on radiata pine seedlings from New Zealand found no infection when the air temperature was less than 7°C or when the leaf wetness period was less than ten hours (Gilmour 1981). The incubation period ranged from 32-107 days, with period length being related to climate as short periods have coincided with warm months (Ivory 1972). The amount of rainfall over a certain time of the year, such as from June to September, can also be a good indicator of infection (Peterson 1973).

In BC, Woods *et al.* (2005) noticed a strong spatial correlation between increased mean summer precipitation (MSP) and infection by *D. septosporum* within the area of the current outbreak. Temperature, relative humidity, and leaf wetness thresholds conducive to the development of Dothistroma here in BC are yet to be documented.

1.4.2 Topography

Site characteristics such as aspect, elevation, slope, and slope position may all have an effect on disease severity. In the northern hemisphere, a north-facing slope may receive much less solar radiation than a south-facing slope, resulting in cooler and moister conditions

on the north-facing slope (Spurr and Barnes 1980). East- and west-facing slopes show a similar trend, but to a lesser extent. East-facing slopes are exposed to direct sunlight in the morning, and are normally cooler and moister than west-facing slopes which receive maximum sunlight in the afternoon (Spurr and Barnes 1980). The effect of aspect on microclimatic conditions thus may produce variability in the development of fungal pathogens, and has been noted with diseases such as Sirococcus shoot blight, dogwood anthracnose, and Swiss needle cast. Anglberger and Halmschlager (2003) found that the intensity of Sirococcus shoot blight in spruce was highest on west exposed upper slopes as well as on hilltops. Dogwood anthracnose symptoms were found to be most severe on trees growing on slopes with north aspects, and least severe on east aspects (Windham *et al.* 1993). In the case of Swiss needle cast, caused by the fungus *Phaeocryptopus gaeumannii* (Rhode) Petrak, fog occurrence, precipitation, temp, elevation, and slope aspect were all variables that contributed to explain most of the variability in disease severity (Rosso and Hansen 2003). Manter *et al.* (2003) also noted that plots with southern exposures, which received greater amounts of solar radiation, had greater amounts of needle abscission compared to north-aspect plots with similar amounts of *P. gaeumannii* colonization. Any influence of aspect on severity of Dothistroma infection has yet to be documented.

Microclimatic conditions can also be influenced by elevation, slope, and slope position. Areas of higher elevation have lower average temperatures than areas of lower elevation (Kimmins 1997). However cool temperatures can occur at low elevations in small depressions and openings where cool air can pool (Kimmins 1997). The influence of elevation and slope on Dothistroma severity has been noted previously. Turner and Lambert (1978) found that Dothistroma severity increased along higher slopes, with lower and more

variable infection on lower slopes. Cobb and Miller (1968) found that Dothistroma appeared to be less severe in mixed stands of knobcone and radiata pine, but they also speculated that this could have been due to microclimatic conditions, i.e. knobcone pine was planted on a slope above the other pines. Marks and Hepworth (1986) found 'hot spots' of Dothistroma in depressions, shallow gullies, and flat land; in areas where cool air could pool. Trees in the path of freely draining cool air showed only very light infection. No disease was noted on higher ridge tops, and was observed infrequently on middle and upper slopes.

1.4.3 Proximity to water

The transport of *D. septosporum* conidia passively through water droplets has been noted in previous literature (Ivory 1972; Bradshaw 2004). Work by Gadgil (1974) found that infection severity was higher and the pre-reproductive period was shorter in foliage kept continuously wet than when it was kept wet for 8, 24, or 48 hours after inoculation. This effect has been noted with other diseases such as dogwood anthracnose, in which leaf wetness was positively associated with greater disease severity (Windham *et al.* 1993). Another study on dogwood anthracnose also found that disease incidence and severity were greatest in the canopies of understory trees where evaporative potential was lowest (Chellemi and Britton 1992). In the case of a pitch canker caused by the fungus *Fusarium circinatum* Nirenberg and O'Donnell infecting radiata pine, Wikler *et al.* (2003) found that the reduced evaporative demand associated with longer periods of intense fog in the coastal zone, as opposed to the inland zone, may increase the efficiency of the infection process. Similarly heavy fogs common to low-lying pine plantations in close proximity to rivers, lakes, or streams may facilitate Dothistroma development and thereby contribute to disease severity.

1.5 Research questions

Knowledge about the timing of *D. septosporum* symptom development and reproduction in BC has previously come from observations on Vancouver Island (Parker and Collis 1966). The most recent studies on the Dothistroma needle blight outbreak in northwestern BC have identified a clear mechanistic relationship between an observed climate trend and the host-pathogen interaction, established an outbreak history for the past two centuries, and determined that genetic diversity of the pathogen population is high (Woods *et al.* 2005; Welsh 2007; Dale 2008). Although weather and topographic conditions are known have to a significant influence on Dothistroma disease severity, they are yet to be described in detail. Further investigation may also provide evidence for the roles of sexual reproduction in the current outbreak as well as the toxin dothistromin in pathogenicity.

This study addresses several important questions concerning the disease development dynamics of Dothistroma needle blight in lodgepole pine. Specifically:

- What weather variables drive *D. septosporum* reproduction and Dothistroma needle blight development?
- How frequent is the sexual stage of *D. septosporum* (= *Mycosphaerella pini* Rost. in Munk) in northwestern BC?
- How do site factors (e.g. elevation, slope, aspect) and nearby water bodies affect disease intensity, and can their influence be explained by microclimate requirements for *D. septosporum* reproduction and Dothistroma needle blight development?

1.6 Study objectives

The specific objectives of this study were as follows:

- To relate Dothistroma disease development to temperature, relative humidity, and leaf wetness conditions.
- To determine the frequency and timing of the sexual stage, *M. pini*, relative to the asexual stage, *D. septosporum*.
- To quantify variation in disease severity relative to site factors (higher vs. lower elevations, slope, slope position, aspect).
- To determine whether disease distribution and severity are influenced by close proximity to major water bodies (e.g. rivers, lakes).

1.7 Literature cited

Ades, P. K., and Simpson, J. A. 1990. Clonal selection for resistance to Dothistroma needle blight in *Pinus radiata*. New Forests 4(1):27-35.

Agrios, G. N. 1997. Plant pathology. Academic Press. 4th ed. San Diego, California, USA.

Alzamora, R. M., Hauer, P., and Peredo, H. 2004. Evaluation of commercial volume losses to *Pinus radiata* caused by *Dothistroma septospora* under varying forest management and chemical control conditions in the province of Valdivia. Bosque 25(1):15-27.

Ames, B. N., Magaw, R., and Gold, L.S. 1987. Ranking possible carcinogenic hazards. Science 236:271-279.

Anglberger, H., and Halmschlager, E. 2003. The severity of *Sirococcus* shoot blight in mature Norway spruce stands with regard to tree nutrition, topography and stand age. Forest Ecology and Management 177:221-230.

Barnes, I., Kirisits, T., Akulov, A., Chhetri, D. B., Wingfield, B. D., Bulgakov, T. S., and Wingfield, M. J. 2008. New host and country records of the Dothistroma needle blight pathogens from Europe and Asia. Forest Pathology 34(3):163-185.

Barnes, I., Crous, P. W., Wingfield, B. D., and Wingfield, M. J. 2004. Multigene phylogenies reveal that red band blight is caused by two distinct species of *Dothistroma*, *D. septosporum* and *D. pini*. Studies in Mycology 50:551-565.

Bednarova, M., Bodejckova, I., Palovcíková, D. and Jankovský, L. 2007. The Contemporary Situation of Dothistroma Needle Blight Outbreak in the Czech Republic. Acta Silvatica et Lignaria Hungarica Special Edition 2007:17-21.

Bradshaw, R. E. 2004. Dothistroma (red-band) needle blight of pines and the dothistromin toxin: a review. Forest Pathology 34(3):163-185.

Bradshaw, R. E., Ganley, R. J., Jones, W. T., and Dyer, P. S. 2000. High levels of dothistromin toxin produced by the forest pathogen *Dothistroma pini*. Mycological Research 104(3):325-332.

Brown, A., Rose, D., and Webber, J. 2003. Red band needle blight of pine. Pages 1-6 in: Forestry Commission Information Note 49.

Bulman, L. S. 1993. Cyclaneusma needle-cast and Dothistroma needle blight in NZ pine plantations. New Zealand Journal of Forestry 38(2):21-24.

Bulman, L. S., Gadgil, P. D., Kershaw, D. J., and Ray, J. W. 2004. Assessment and control of Dothistroma needle-blight. New Zealand Forest Health Research Collaborative, Forest Research Bulletin 229.

Butin, H. 1995. Tree Diseases and Disorders. Oxford University Press, New York.

Butin, H., and Richter, J. 1983. Dothistroma needle blight: a new pine disease in the Federal Republic of Germany. Nachrichtenbl. Deut. Pflanzenschutzd. 35:129-131.

Carson, S. D. 1989. Selecting *Pinus radiata* for resistance to Dothistroma needle blight. New Zealand Journal of Forestry Science 19(1):3-21.

Chellemi, D. O., and Britton, K. O. 1992. Influence of canopy microclimate on incidence and severity of dogwood anthracnose. Canadian Journal of Botany 70:1093-1096.

Cobb, F. W., and Miller, D. R. 1968. Hosts and Geographic Distribution of *Scirrhia pini* - The Cause of the Red Band Needle Blight in California. Journal of Forestry 66(12):930-933.

Coops, N., Stanford, M., Old, K., Dudzinski, M., Culvenor, D., and Stone, C. 2003. Assessment of Dothistroma Needle Blight in *Pinus radiata* Using Airborne Hyperspectral Imagery. The American Phytopathological Society 93:1524-1532.

Dale, A. 2008. Dothistromin production and its relationship to genetic diversity in the fungal pathogen *Dothistroma septosporum*. M.S. thesis. University of Northern British Columbia, Prince George.

Dick, A. M. P. 1989. Control of Dothistroma needle blight in the *Pinus radiata* stands of Kinleith Forest. New Zealand Journal of Forestry Science 19(2-3):171-179.

Elliott, G. S., Mason, R. W., Ferry, D. G., and Edwards, I. R. 1989. Dothistromin risk assessment for forestry workers. New Zealand Journal of Forestry Science 19(2/3):163-170.

[EMPPO] European and Mediterranean Plant Protection Organization. 2005. Diagnostics: *Mycosphaerella pini*. EPPO Bulletin 35:303-306.

Evans, H. 1984. The genus *Mycosphaererella* and its anamorphs *Cercoseptoria*, *Dothistroma* and *Lecanosticta* on pines. Mycological Papers 153:1-102.

FIDs. 1984, 1985, 1986. Forest insect and disease conditions, Prince Rupert Forest Region.

Franich, R. A. 1988. Chemistry of weathering and solubilisation of copper fungicide and the effect of copper on germination, growth, metabolism, and reproduction of *Dothistroma pini*. New Zealand Journal of Forestry Science 18(3):318-328.

Franich, R. A., Carson, M. J., and Carson, S. D. 1986. Synthesis and accumulation of benzoic acid in *Pinus radiata* needles in response to tissue injury by dothistromin, and correlation with resistance of *P. radiata* families to *Dothistroma pini*. Physiological and Molecular Plant Pathology 28:267-286.

Funk, A. 1979. Sexuality in *Scirrhia pini*. Canadian Forestry Service Bi-monthly Research Notes 35:14.

Funk, A. 1985. Foliar Fungi of Western Trees. Ministry of Supply and Services Canada. Victoria, Canada.

Gadgil, P. D. 1977. Duration of leaf wetness periods and infection of *Pinus radiata* by *Dothistroma pini*. New Zealand Journal of Forestry 7(1):83-90.

Gadgil, P. D. 1974. Effect of temperature and leaf wetness period on infection of *Pinus radiata* by *Dothistroma pini*. New Zealand Journal of Forest Science 4:495-501.

Gadgil, P. D. 1970. Survival of *Dothistroma pini* on fallen needles of *Pinus radiata*. New Zealand Journal of Botany 8:303-9.

Gadgil, P. D. 1967. Infection of *Pinus radiata* needles by *Dothistroma pini*. New Zealand Journal of Botany 5:498-503.

Gadgil, P. D., and Holden, G. 1976. Effect of light intensity on infection of *Pinus radiata* by *Dothistroma pini*. New Zealand Journal of Forestry Science 6(1):67-71.

Gibson, I. A. S. 1974. Impact and control of Dothistroma blight of pines. European Journal of Forest Pathology 4:89-100.

Gibson, I. A. S. 1972. Dothistroma blight of *Pinus radiata*. Annual Review of Phytopathology 10:51-72.

Gibson, I. A. S., Christensen, P. S., and Munga, F. N. 1964. First observations in Kenya on a foliage disease of pines caused by *Dothistroma pini* Hulbary. Commonwealth Forestry Review 43:31-48.

Gilmour, J. W. 1981. The effect of season on infection of *Pinus radiata* by *Dothistroma pini*. European Journal of Forest Pathology 11:265-269.

Gilmour, J. W., and Noorderhaven, A. 1971. Influence of time of application of cuprous oxide on control of Dothistroma needle blight. New Zealand Journal of Forestry Science 1(2):160-166.

Gilmour, J.W., and Crockett, F. 1972. *Dothistroma pini* project: monitoring of infection patterns in the field. Forest Research Institute Report No. 53. New Zealand Forest Service.

Groenewald, M., Barnes, I., Bradshaw, R. E., Brown, A. V., Dale, A., Groenewald, J. Z., Lewis, K. J., Wingfield, B. D., Wingfield, M. J., and Crous, P. W. 2007. Characterization and Distribution of Mating Type Genes in the Dothistroma Needle Blight Pathogens. Phytopathology 97(7):825-834.

Harrington, T. C., and Wingfield, M. J. 1998. Diseases and the ecology of indigenous and exotic pines. Pages 381-401 in: Richardson DM, editor. Ecology and biogeography of *Pinus*. Cambridge University Press. Cambridge, UK.

Harris, J. M., and McConchie, D. L. 1978. Wood properties of *Pinus radiata* infected with *Dothistroma pini*. New Zealand Journal of Forestry Science 9(3):410-416.

Hunt, R. S. 1981. Pine needle cast and blights in the Pacific Region. Canadian Forest Service. Pacific Forest Research Center, Forest Pest Leaflet No. 43:1-7.

Ivory, M. H. 1994. Records of foliage pathogens of *Pinus* species in tropical countries. Plant Pathology 43(3):511-518.

Ivory, M. H. 1972. Infection of *Pinus radiata* foliage by *Scirrhia pini*. Transactions of the British Mycological Society 59(3):365-375.

Ivory, M. H. 1967. A new variety of *Dothistroma pini* in Kenya. Transactions of the British Mycological Society 50(2):289-297.

Karadžić, D. M. 1994. *Picea omorika* - a new host of *Dothistroma septospora*. European Journal of Forest Pathology 24:300-303.

Karadžić, D. 1989. *Scirrhia pini* Funk et Parker. Life cycle of the fungus in plantations of *Pinus nigra* Arn. In Serbia. European Journal of Forest Pathology 19:231-236.

Kimmins, J. P. 1997. Forest Ecology: A Foundation for Sustainable Management. Prentice-Hall Inc. 2nd ed. Upper Saddle River, New Jersey, USA.

Lotan, J. E., and Critchfield, W. B. 1990. *Pinus contorta* Dougl. ex. Loud. Lodgepole Pine. Pages 302-315 in: Agriculture Handbook 654. Silvics of North America. Washington, USA.

Manter, D. K., Winton, L. M., Filip, G. M., and Stone, J. K. 2003. Assessment of Swiss Needle Cast Disease: Temporal and Spatial Investigations of Fungal Colonization and Symptom Severity. Journal of Phytopathology 151(6):344-351.

Marks, G. C., and Hepworth, G. 1986. Effect of Certain Features of a Stand of *Pinus radiata* on *Dothistroma septospora* Needle Blight. Australian Forest Research 16:223-229.

McGarrity, K., and Hoberg, G. 2005. The beetle challenge: An overview of the mountain pine beetle epidemic and its implications. Issue Brief, Forest Policy Resources website. Available from: http://policy.forestry.ubc.ca/issuebriefs/pinebeetle.html Accessed June 22 2008.

Muir, J. A., and Cobb, F. W. 2005. Infection of radiata and bishop pine by *Mycosphaerella pini* in California. Canadian Journal of Forest Research 35(11):2529-2538.

[NRC] Natural Resources Canada. 2003. CTD - Conifer Needle Diseases: Dothistroma Needle Blight. Available from:

http://www.pfc.forestry.ca/diseases/CTD/Group/Needle/needle5_e.html Accessed March 3 2006.

Oliver, R., and Schweizer, M. 1999. Molecular Fungal Biology. Cambridge University Press. Cambridge, UK.

Parker, A. K. 1972. Artificial inoculation of *Pinus radiata* with *Scirrhia* (*Dothistroma*) *pini*: effect of relative humidity and temperature on incubation. Phytopathology 62:1160-1164.

Parker, A. K., and Collis, D. G. 1966. Dothistroma needle blight of pines in British Columbia. Forestry Chronicle 30:160-161.

Pedersen, L. 2004. How Serious is the Mountain Pine Beetle Epidemic? From a Timber Supply Perspective. Available from: http://www.for.gov.bc.ca/hfd/library/MPB/pederson_2004_how.pdf Accessed June 22 2008.

Peterson, G. W. 1982. Dothistroma Needle Blight of Pines. Available from: http://www.na.fs.fed.us/spfo/pubs/fidls/dothistroma/doth.htm Accessed March 3 2006.

Peterson, G. W. 1981. Page 133 in: Research in the United States on Dothistroma needle blight of pines. Proceedings: Conference of the IUFRO working party on needle diseases. Sarajevo, Yugoslavia.

Peterson, G. W. 1973. Infection of Austrian and Ponderosa Pines by *Dothistroma pini* in Eastern Nebraska. Phytopathology 63:1060-1063.

Peterson, G. 1969. Dothistroma needle blight of Austrian and ponderosa pines: epidemiology and control. Phytopathology 57:437-441.

Peterson, G. W. 1966. Penetration and infection of Austrian and ponderosa pines by *Dothistroma pini*. Phytopathology 56:894-895.

Peterson, G. H., and Walla, J. A. 1978. Development of *Dothistroma pini* upon and within needles of Austrian and ponderosa pines in eastern Nebraska. Phytopathology 68:1422-1430.

Phillips, D. H., and Burdekin, D. A. 1982. Diseases of Forests and Ornamental Trees. MacMillan Press Ltd. London, UK.

Province of British Columbia. 2007. Annual Report of the Ministry of Forests, 1996-1997. Available from: http://www.for.gov.bc.ca/mof/annualreports.htm Accessed February 16 2007.

Province of British Columbia. 2001. Timber Supply Review [Kispiox TSR]. Available from: http://www.for.gov.bc.ca/hts/tsa/tsa12/docs.htm Accessed March 7 2007.

Rosso, P. H., and Hansen, E. M. 2003. Predicting Swiss Needle Cast Disease Distribution and Severity in Young Douglas-Fir Plantations in Coastal Oregon. Phytopathology 93:790-799.

Schwelm, A., Barron, N. J., Baker, J., Dick, M., Long, P.G., Zhang, S., and Bradshaw, R.E. 2009. Dothistromin toxin is not required for dothistroma needle blight in *Pinus radiata*. Plant Pathology 58:293-304.

Shain, L., and Franich, R. A. 1981. Induction of Dothistroma blight symptoms with dothistromin. Physiological Plant Pathology 19:49-55.

Spurr, S. H., and Barnes, B. V. 1980. Forest Ecology. Krieger Publishing Company. 3rd ed. Malabar, Florida.

Stoessl, A., Abramowski, Z., Lester, H. H., Rock, G. L., and Towers, G. H. N. 1990. Further toxic properties of the fungal metabolite dothistromin. Mycopathologia 112:179-186.

Taylor, J. E., and Schwandt, J. W. 1998. Dothistroma needle blight of limber pine in Montana. USDA Forest Service Report 98-4.

Taylor, J. E., and Walla, J. A. 1999. First Report of *Dothistroma septospora* on Native Limber and Whitebark Pine in Montana. Plant Disease 83(6):590.

Turner, J., and Lambert, M. J. 1978. Pages 546-564 in: Sulphur nutrition of conifers in relation to response to fertilizer nitrogen, to fungal infections and to soil parent materials. Proceedings of the fifth North American Forest Soil Conference. Colorado, USA.

Van der Pas, J. B. 1981. Reduced early growth rates of *Pinus radiata* caused by *Dothistroma pini*. New Zealand Journal of Forestry Science 11(3):210-220.

Van der Pas, J. B., Kimberley, M. O., and Kershaw, D. J. 1984. Evaluation of the assessment of Dothistroma needle blight in stands of *Pinus radiata*. New Zealand Journal of Forestry Science 14(1):3-13.

Welsh, C. 2007. The relationship between climate and outbreak dynamics of Dothistroma needle blight in northwest British Columbia, Canada. M.S. thesis. University of Northern British Columbia, Prince George.

Whyte, A. G. D. 1969. Tree growth in the presence of *D. pini*. Pages 51-53 in: Forest Research Institute Annual Report for 1968. New Zealand Forest Service.

Wikler, K., Storer, A. J., Newman, W., Gordon, T. R., and Wood, D. L. 2003. The dynamics of an introduced pathogen in a native Monterey pine (*Pinus radiata*) forest. Forest Ecology and Management 179:209-221.

Windham, M. T., Montgomery-Dee, M. E., and Parham, J. 1993. Pages 184-185 in: Site Parameters That Affect Dogwood Anthracnose Incidence and Severity. Proceedings of the SNA research conference thirty-eighth annual report. McMinnville, USA.

Woods, A. J. 2003. Species diversity and forest health in northwest British Columbia. The Forestry Chronicle 79(5):892-897.

Woods, A., Coates, K. D., and Hamann, A. 2005. Is an Unprecedented Dothistroma Needle Blight Epidemic Related to Climate Change? Bioscience 55(9):761-769.

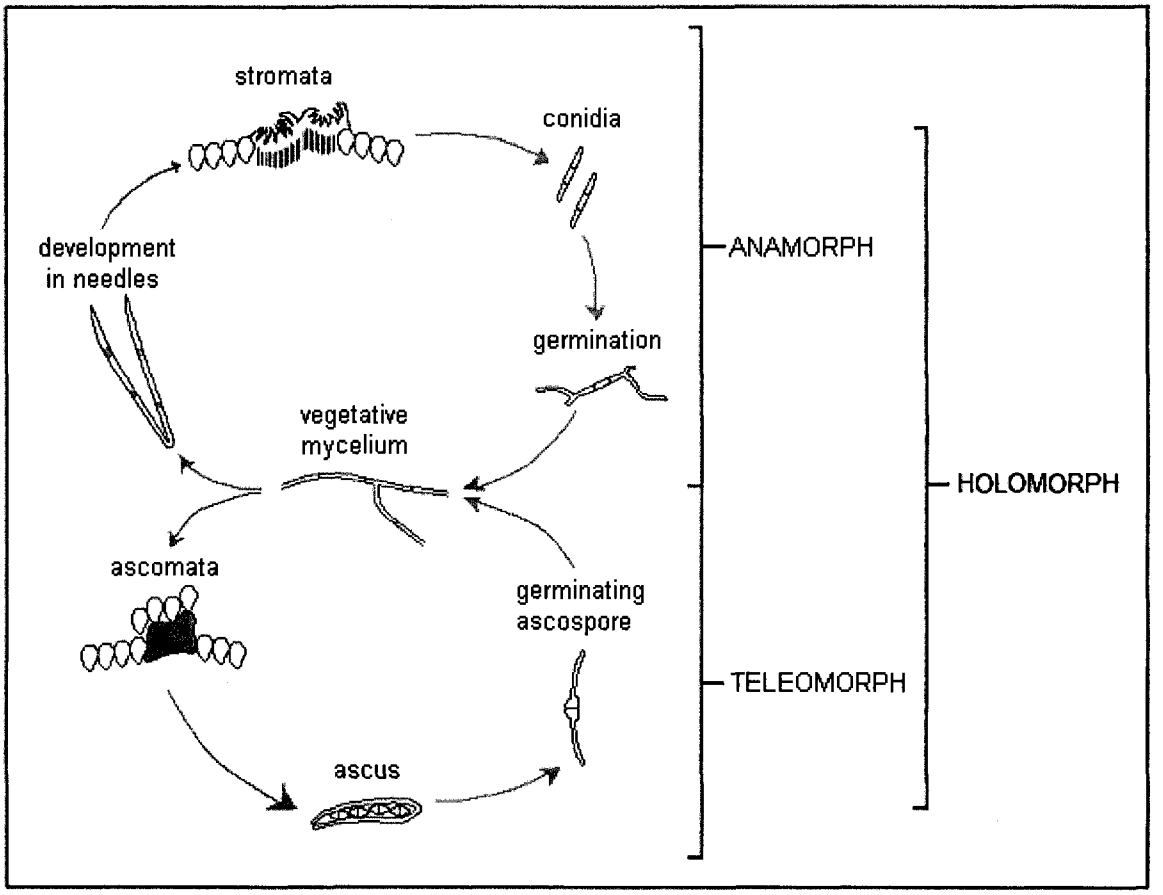

Figure 1.1. Life cycle of *Dothistroma septosporum* (anamorph) and *Mycosphaerella pini* (teleomorph and holomorph). Adapted from Butin (1995) and Funk (1985).

CHAPTER 2: METHODS

2.1 Study 1: Plot monitoring and weather analysis methods

2.1.1 Study area description

This study was conducted in northwestern British Columbia (BC) in the Kispiox Timber Supply Area (TSA) of the Skeena Stikine Forest District. Though the Skeena Stikine Forest District is primarily Sub-boreal spruce (SBS), the majority the Kipsiox TSA is classified as Interior Cedar-Hemlock (ICH) – one of the wettest interior biogeoclimatic zones (Stevens 1995). Its low- to mid-elevation forests have a climate that is warm and moist in the summer, cool and wet in the fall, and cold in the winter (Banner *et al.* 1993). Western hemlock (*Tsuga heterophylla* [Raf.] Sarg.), western redcedar (*Thuja plicata* Donn ex D. Don), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), and Lutz spruce (*Picea glauca* [Moench] Voss x *Picea sitchensis* Bong. Carrière, also known as Roche spruce in BC interior) comprise the climax forests of the ICH (Banner *et al.* 1993). Over the past two to three decades forest management practices have caused a species shift towards interior spruce (*Picea engelmannii* Parry ex Englem. x *Picea glauca*) and lodgepole pine (*Pinus contorta* Douglas & Loud. var *latifolia* Engelm ex S. Wats.) in this area (Woods 2003). More than 40,000 ha of young lodgepole pine now exist in the Kispiox TSA, representing approximately 40% of managed stands as compared to a historical level of 10% (Woods *et al.* 2005). The current Dothistroma needle blight epidemic has centered over the Kispiox Timber Supply Area (TSA).

2.1.2 Site selection

Four sites were selected from three TSAs within the Skeena Stikine Forest District: Bulkley, Kispiox, and Cranberry (see Figure 2.1). Within each site, three lodgepole pine

stands were selected that had signs of Dothistroma infection, were accessible, and were between 10-25 years of age. Stands were a minimum of 4 km apart, and at least 50 m from the nearest road. One plot of 5.64 m radius was established per stand; therefore a total of twelve plots were established for weekly monitoring. Universal Transverse Mercator (UTM) coordinates and elevations for all plot centers were obtained using a handheld GPS unit (© GARMIN Corp.). Aspect, slope and slope position were also recorded using a handheld compass and clinometer.

Weather stations were positioned in the centre of each plot on a mounting pole approximately one meter off the ground and housed inside protective solar radiation shields. The one meter height of the weather stations was close to the height of the live crown threshold on candidate trees. Six plots from the Cranberry and Helen Lake sites were equipped with HOBOs (HOBO Pro Series, © Onset Corp.), and six plots from the Muldoe and Bulkley sites were equipped with microclimate data loggers (Micro Station Data Logger H21-002, © Onset Corp.). At all twelve plots temperature (°C) and relative humidity (%) were recorded at 15-minute intervals from early summer (June 1, 2008) to fall (Sept 30, 2008). Plots with data loggers also recorded leaf wetness (%) at 15-minute intervals. Weather variables to be recorded were determined from previous studies (Gadgil 1974; Gibson 1974; Gilmour 1981; Woods *et al.* 2005).

2.1.3 Tree and needle selection

All lodgepole pine trees within a 5.64 m radius of the plot center were measured for diameter at breast height, tree height, live crown height, and functional live crown according to the Defoliation Free Growing Damage Standard for Determinate Growth Conifers (BCMOFR 2005). Six trees were selected from those within the plot. On each tree four

nodes, each representing an annual cohort of needles, were selected from the lower range of live crown and flagged. Removable bags made of black screen-door material were fit to each node in order to retain senescing needles. Ten one-year-old needles were selected from each node and were marked with blue paint on the distal tip. All selections were random.

2.1.4 Symptom observations

Three categories of disease expression were recorded: red banding, fruiting body development, and spore production. Bands were considered to be symptoms of *D. septosporum* infection if they were red and had an abrupt transition zone between healthy and diseased needle tissue (see Figure 2.2). Fruiting bodies were considered to be *D. septosporum* based on color, shape, and emergence, as described by Funk (1985) and EMPPO (2005). When fruiting bodies were detected, two procedures were followed: (1) marked needles bearing fruiting bodies were dipped in a 3 mL aliquot of distilled water in order to capture spores (referred to as the needle dip method), and (2) ten unmarked needles were collected for closer examination and fruiting body dissection.

2.1.5 Laboratory methods

Spore counting. From each 3 mL aliquot 10 µL of fluid was inserted into a hemacytometer (Hausser Bright-Line, © 2007 Hausser Scientific) using a pipette with disposable pipette tips. After an aliquot had been sampled, the pipette tip was disposed. All spores in each of the four 1 mm x 1 mm squares were counted. Spores touching the top or left borders were counted. Spores touching the bottom or right borders were not counted. The spore count was calculated using the following formula in order to obtain a relative measure of spore production:

$$(\text{number of spores/square}) \times 10^4 = \text{spores/mL}$$

The use of a hemacytometer for spore counting has been used previously in work on fungal diseases such as *Sphaeropsis sapinea* (causal agent of Sphaeropsis tip blight) and *Botrytis cinerea* (causal agent of Botrytis blossom blight) (Blodgett and Stanosz 1997; Smith 1998). This procedure was adapted from Amberg *et al.* (2005). Spores were identified based on descriptions by Funk (1985) and EMPPO (2005).

Fruiting body dissection. Under a dissecting microscope *D. septosporum* fruiting bodies were extracted from needle tissue, prepared in 10% KOH, and examined under a compound microscope to determine whether they were stromata (asexual) or ascomata (sexual). The presence of conidia and ascospores were used to confirm the reproductive stage of the fruiting body. Spores and fruiting bodies were identified based on descriptions by Funk (1985) and EMPPO (2005).

2.1.6 Statistical methods

Field data was compiled into spreadsheet format using EXCEL (Office Excel, © 1985-2003 Microsoft Corp.). Paired two-sample *t*-tests were used to compare daily mean weather variables between Dothistroma needle blight signs and symptoms. Daily mean weather variables between days Dothistroma needle blight signs were and were not observed were also compared using paired two-sample *t*-tests. Mixed effects models were used to assess the effects of temperature, relative humidity, and leaf wetness on banding and fruiting body development. A logistic regression model with mixed effects was used to assess the effects of temperature, relative humidity, and leaf wetness on spore production. For all models site, plot, tree, and node were treated as random effects. Time, as measured in Julian days, and sample size (number of needles) were treated as fixed effects. All models were

constructed using the statistical software R 2.4.1 (Ihaka and Gentleman 1996; R Development Core Team 2004).

Weather variables were examined above various thresholds over intervals of 2, 4, 7, and 14 consecutive days. Temperature (°C) was tested above the following daily minimum, maximum, and mean thresholds: 6, 7, 8, 9, 10, 12, 14, 16, and 18. Relative humidity (%) was tested above the following daily minimum, maximum, and mean thresholds: 60, 70, 80, and 90. Leaf wetness (%) was tested above the following daily minimum, maximum, and mean thresholds: 30, 40, 50, 60, 70, 80, and 90. Based on field observations, leaf wetness values of $\geq 30\%$ indicate the presence of liquid water on pine needles. All leaf wetness analyses were restricted to the six plots equipped with leaf wetness sensors. Weather variables were determined to be significant if the p-value of the *t*-test was less than or equal to 0.05. Backwards selection was used to create models for the red banding, fruiting body, and spore production symptoms. This process involved fitting models with all weather variables, removing the least significant variable, re-fitting the model, and repeating until only significant variables ($p < 0.05$) remained. 2- and 14-day models aided in determining the most consistent weather variables to retain in 4- and 7-day models. After graphical inspection of the residual plots, square root transformations were used for the red banding and fruiting body models to meet analytical assumptions (homoscedasticity of variances and normal distribution of residuals).

2.2 Study 2: Topographic analysis methods

2.2.1 Study area description

This study was conducted in northwestern BC across two Forest Districts: the Skeena Stikine and the Kalum. Aerial survey data from 2006 provided by the Ministry of Forests and

Range in Smithers, BC, was used to investigate topographical effects on disease development. The aerial survey data followed the results of an earlier survey in 2002, which assessed the extent of damage due to this epidemic in the Kispiox and Cranberry TSAs. The aerial survey procedures were jointly developed by Paul Hanna and Alex Woods (Alex Woods[1], personal communication, February 4, 2009). In that survey all lodgepole pine leading stands were assessed using a low-level aerial overview, covering a total area of 21,564 ha. Stands were assessed for both the degree of Dothistroma-induced defoliation and the amount of alternate conifer species stocking.

The 2006 aerial survey covered 38,787 ha of managed stands from the following five TSAs: Bulkley, Cranberry, Kalum, Kispiox, and Nass (see Figure 2.3). This area can be characterized by the biogeoclimatic ecosystem classification (BEC) system, equivalent to the Ecological Land Classification (ELC) System used in other provinces. Used in forestry and conservation, BEC was developed by the BC Ministry of Forests to categorize the high ecosystem variability unique to the province. The ELC system describes fifteen ecozones across Canada, five of which occur in BC (Environment Canada 2005). BEC identifies fourteen zones in BC with descriptions that incorporate three levels of organization: regional, local, and chronological (Meidinger and Pojar 1991). Vegetation, soils, and topography are used to infer regional climate and identify areas that have relatively uniform climate at a regional level (Meidinger and Pojar 1991). At a local level landscape segments are classified into site units that have relatively uniform vegetation, soils, and topography (Meidinger and Pojar 1991). At a chronological level, vegetation units are recognized at a particular site and arranged according to site history and successional status (Meidinger and Pojar 1991). As

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shown in Table 2.1, four BEC zones overlap in the study area: Interior Cedar-Hemlock (ICH), Coastal Western Hemlock (CWH), Sub-boreal spruce (SBS), and Engelmann Spruce - Subalpine Fir (ESSF).

The ICH comprises low- to mid-elevation forests that contain western hemlock, western redcedar, subalpine fir, and Roche spruce. Western hemlock, western redcedar, Sitka spruce (*Picea sitchensis* [Bong.] Carr.), and amabilis fir (*Abies amabilis* Douglas ex Forbes) are also present in the old-growth conifer forests of the CWH. CWH comprises low- to mid-elevation forests, known to have a maritime or oceanic climate with relatively mild temperatures and heavy rainfall (Banner *et al.* 1993). The SBS zone covers most of the interior lowland forest with a climax forest that is dominated by two species: hybrid white spruce (*Picea glauca* [Moench] Voss x *Picea engelmannii* Parry ex Englem.) and subalpine fir. Lodgepole pine and trembling aspen (*Populus tremuloides* Michx.) comprise frequent and extensive seral stands (Banner *et al.* 1993). It has a primarily continental climate, characterized by seasonal extremes of temperature, with severe, snowy winters and relatively warm, moist, and short summers (Banner *et al.* 1993). The ESSF zone covers high-elevation or subalpine forests dominated by subalpine fir, with lodgepole pine present in drier areas (Banner *et al.* 1993). Although long-term climate data is lacking, it is known to have a shorter, cooler, and moister growing season than adjacent low-elevation zones, and a longer, colder, and snowier winter (Banner *et al.* 1993).

2.2.2 Survey criteria

The purpose of the aerial survey was to assess the extent and severity of Dothistroma damage in lodgepole pine leading plantations in the five TSAs, and to provide a list of stands that will require fill-planting as a result of low stocking levels and high Dothistroma

incidence. Dothistroma infection severity was assessed by aerial survey, rated as functional live crown (FLC). Functional live crown (in percent) was calculated as a function of average live crown and average live nodes on a tree.

2.2.3 Disease estimate criterion

Live crown and live node data were collected for each opening. Live crown data were broken into 3 classes: 0-20%, 21-70%, and 71-100%. Live node data were broken into 6 classes: 5, 4, 3, 2, 1, and 0 live nodes. The percentage of pine in each class of the live crown and live node categories was visually estimated. An average live crown was calculated for each opening as a combined weighted average of the 3 live crown classes, using the midpoint of each class to weigh the percent of pine in that class. An average live node was also calculated for each opening as a combined weighted average of the 6 live node classes, using the % live node complement for a healthy tree to weight the percent pine in that class. For each opening a final FLC percentage value was calculated as the product of the average live crown and average live nodes.

Below 5% FLC, trees are not expected to recover from the needle loss incurred by disease. Trees with 10% FLC are considered close to death, although likely to recover should the disease subside (Alex Woods[1], personal communication, May 2, 2008). A 20% FLC threshold was included as it is used at an operational level to determine the free-growing status of a tree. It is also the threshold below which appreciable checks in height and diameter growth rates are observed (Gibson 1974). Compared to a healthy tree, a diseased tree with more than 80% defoliation experiences height growth rates as low as 50% and diameter growth rates as low as 10% (Gibson *et al.* 1964; Gibson 1974). According to the

[1] Alex Woods, Regional Forest Pathologist, Northern Forest Region, 3333 Tatlow Road, Smithers, BC, V0J 2N0, (250) 847-6382.

Free Growing Damage Standard, which covers damage caused by both defoliating insects and foliar diseases, a tree with more than 20% healthy foliage is assumed to have a good chance of maintaining its free-growing status if the foliar disease or defoliating insect event were to subside (BCMOFR 2005). In the exceptional case of Dothistroma needle blight, 50% FLC is the recommended threshold to consider a tree healthy.

2.2.4 Spatial analysis

The 2006 aerial survey data were projected as point data in ArcMap 9.2 (© 1999-2006 ESRI Inc.) in NAD1983 UTM Zone 9. Digital elevation models (DEMs) in 1:250,000 grids (25 m x 25 m resolution) were used to obtain elevation values for each of the pine openings in the survey. From the DEMs slope and aspect rasters were generated and used to extract slope and aspect values for pine openings. River and lake data were obtained as vector data in 1:50,000 grids from GeoGratis (NRC 2008). Spatial joins were performed to acquire distance values between pine openings and the nearest rivers and lakes. Northings and eastings (in UTM coordinates) with quadratic terms were also included to contend with potential spatial autocorrelation in analyses of disease severity. All spatial analyses produced outputs in DBF 4 (dBASE IV) format, which could be viewed as spreadsheets in EXCEL (Office Excel, © 1985-2003 Microsoft Corp.). All outputs were combined in a single dataset and saved as a CSV file (in comma-delimited format).

2.2.5 Statistical methods

Disease severity data were organized into binomial variables (0 = likely to recover, 1 = unlikely to recover) using four FLC thresholds: 5%, 10%, 20%, and 50%. Binary logistic regression models were used to test the significance of topographic variables on the likelihood of recovery of pine-leading stands, using R 2.4.1. Topographic variables were

considered significant if the p-value of the Wald's test was less than or equal to 0.05. Backwards selection was used to create models for recovery status of pine-leading stands using the 5% (y_5), 10% (y_{10}), 20% (y_{20}), and 50% (y_{50}) FLC thresholds. The response variable y is a binary variable indicating whether the stand is unlikely to recover ($y = 1$) or likely to recover ($y = 0$) based on the FLC thresholds. Assuming there are k explanatory variables $x = (x_1, x_2, \ldots, x_k)$, predictions from these models are performed by calculating estimated logit scores (y), and then converting these scores into estimated probabilities using the following equation:

$$p(y) = \frac{e^{(\beta_0 + \beta_1 x_1 + \ldots + \beta_k x_k)}}{[1 + e^{(\beta_0 + \beta_1 x_1 + \ldots + \beta_k x_k)}]}$$

2.2.6 Disease hazard rating system and map creation

Following the results of the logistic regression analyses, a disease hazard rating approach was devised. Two logistic regression models were fed into the Map Algebra tool in ArcMap 9.2 (© 1999-2006 ESRI Inc.) to illustrate how the models could be utilized to produce probability maps for disease severity based on topographic factors. The map covers the Cranberry TSA which was selected because it was a relatively small TSA (761.6 km^2), had a good spread of FLC data (37.2 ± 18.9%), fit onto a single 1:250,000 DEM grid sheet, would require the least processing time as the other candidate TSAs would have required merging DEM grid sheets. Rasters of eastings and northings had to be interpolated from a 1000 x 787 point feature coverage created in EXCEL (Office Excel, © 1985-2003 Microsoft Corp.) in DBF 4 format. The 10% and 20% FLC threshold models were used in generating the map because trees are near death below 10% FLC and 20% FLC is the threshold below which growth cessation occurs (Gibson 1974).

2.3 Literature Cited

Amberg, D. C., Burke, D. J., and Strathern, J. N. 2005. Methods in yeast genetics: a Cold Spring Harbor Laboratory course manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York, USA.

Banner, A., MacKenzie, W., Haeussler, S., Thomson, S., Pojar, J., and Trowbridge, R. 1993. A Field Guide to Site Identification and Interpretation for the Prince Rupert Forest Region. British Columbia Ministry of Forests. Victoria, Canada.

[BCMOFR] British Columbia Ministry of Forests and Range. 2006. Dothistroma monitoring program aerial survey data.

[BCMOFR] British Columbia Ministry of Forests and Range. 2005. Defoliation Free Growing Standard for Determinate Growth Conifers. Available from: www.for.gov.bc.ca/HFP/health/fhdata/defoliation_FG.pdf Accessed October 23 2006.

Blodgett, J. T., and Stanosz, G. R. 1997. Differential inhibition of *Sphaeropsis sapinea* morphotypes by a phenolic compound and several monoterpenes of red pine. Phytopathology 87:606-609.

[EMPPO] European and Mediterranean Plant Protection Organization. 2005. Diagnostics: *Mycosphaerella pini*. EPPO Bulletin 35:303-306.

Environment Canada. 2005. Canada's Ecozones and Ecoregions. Available from: http://www.ec.gc.ca/soer-ree/English/Framework/NarDesc/canada_e.cfm Accessed February 24 2009.

Funk, A. 1985. Foliar Fungi of Western Trees. Ministry of Supply and Services Canada. Victoria, Canada.

Gadgil, P. D. 1974. Effect of temperature and leaf wetness period on infection of *Pinus radiata* by *Dothistroma pini*. New Zealand Journal of Forestry Science 4:495-501.

Gibson, I. A. S. 1974. Impact and control of Dothistroma blight of pines. European Journal of Forest Pathology 4:89-100.

Gibson, I. A. S., Christensen, P. S., and Munga, F. N. 1964. First observations in Kenya on a foliage disease of pines caused by *Dothistroma pini* Hulbary. Commonwealth Forestry Review 43:31-48.

Gilmour, J. W. 1981. The effect of season on infection of *Pinus radiata* by *Dothistroma pini*. European Journal of Forest Pathology 11:265-269.

Ihaka, I., and Gentleman, R. 1996. R: a language for data analysis and graphics. Journal of Computational and Graphical Statistics 5(3):299–314.

Meidinger, D. V., and Pojar, J. 1991. Ecosystems of British Columbia. Research Branch, British Columbia Ministry of Forests Special Report Series No. 6. Victoria, British Columbia, Canada.

[NRC] Natural Resources Canada. 2008. GeoGratis – Download Directory. Available from: http://www.geogratis.ca/geogratis/en/download/topographic.html Accessed April 1 2008.

R Development Core Team. 2004. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-90051-00-3 (http://www.R-pro-ject.org).

Smith, B. J. 1998. Botrytis blossom blight of southern blueberries: Cultivar susceptibility and effect of chemical treatments. Plant Disease 82:924-927.

Stevens, V. 1995. Wildlife diversity in British Columbia: distribution and habitat use of amphibians, reptiles, birds, and mammals in biogeoclimatic zones. Res. Br., B.C. Min. For., Wildl. Br., B.C. Min. Environ. Lands and Parks, Victoria, B.C. Work Pap. 04/1995.

Woods, A. J. 2003. Species diversity and forest health in northwest British Columbia. The Forestry Chronicle 79(5):892-897.

Woods, A., Coates, K. D., and Hamann, A. 2005. Is an Unprecedented Dothistroma Needle Blight Epidemic Related to Climate Change? Bioscience 55(9):761-769.

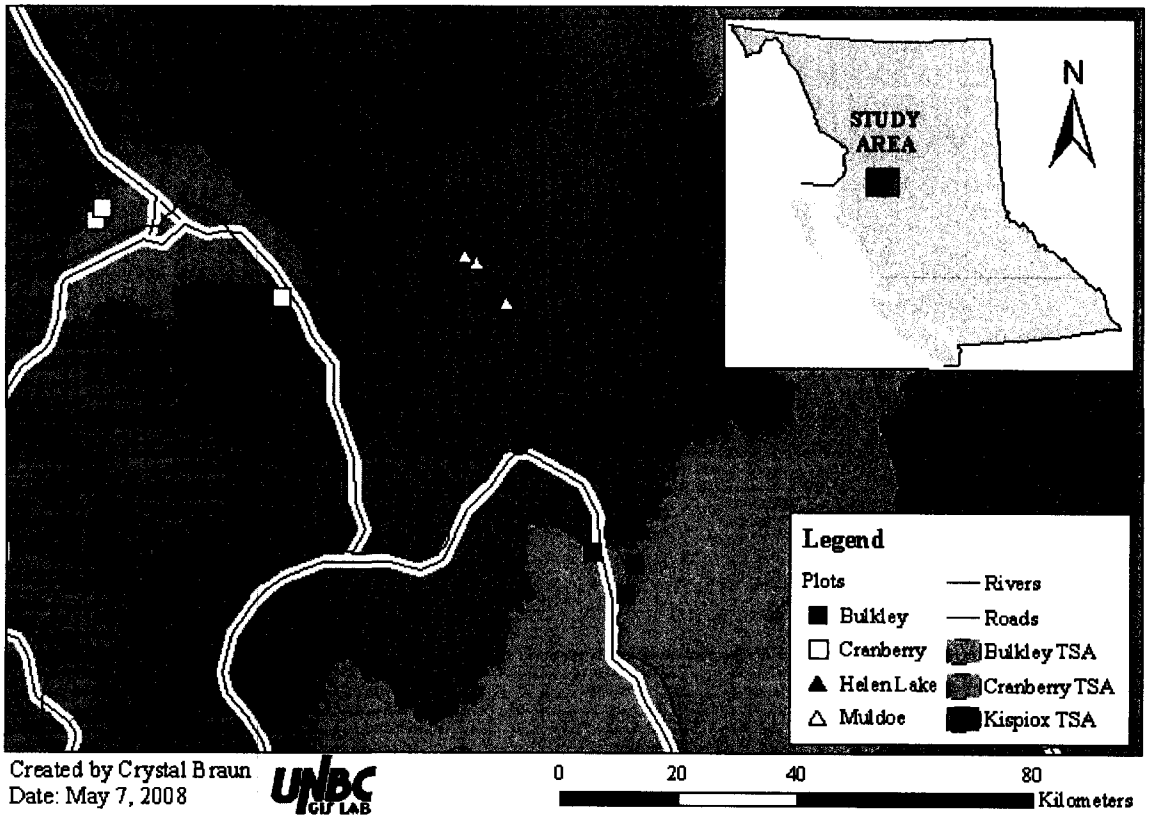

Figure 2.1. Distribution of plots for weather monitoring in the Skeena Stikine Forest District for 2007.

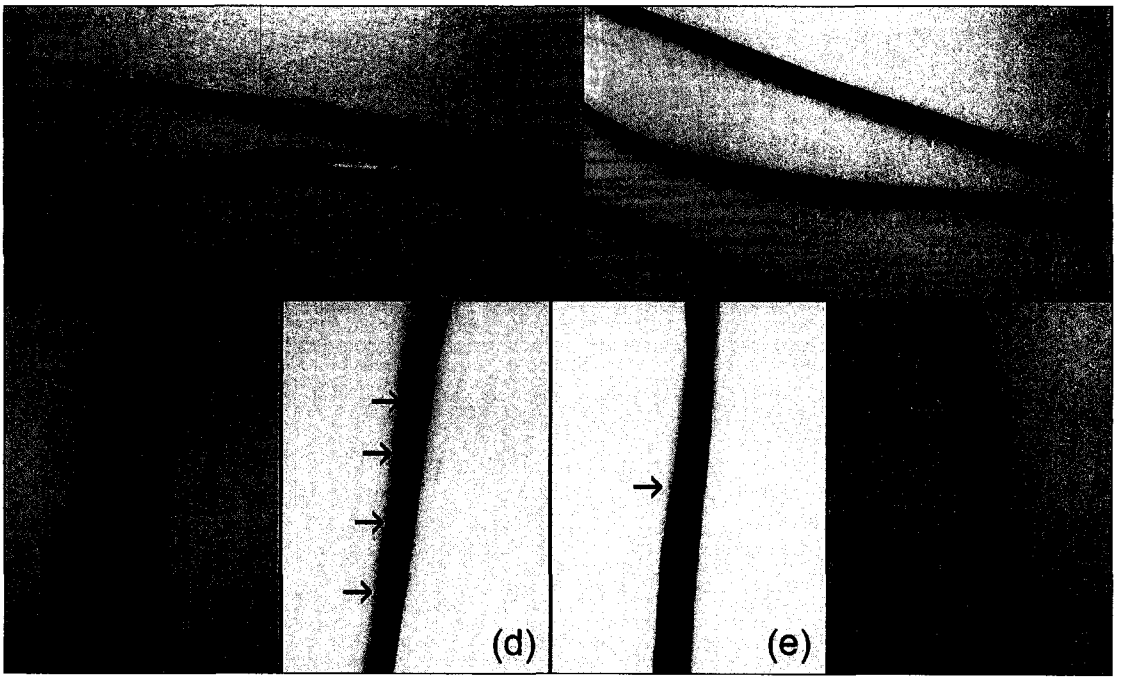

Figure 2.2. Variation in *D. septosporum* banding on lodgepole pine needles. A: asymptomatic green needle infected with *D. septosporum.* B: needles bearing new infections which have either senesced or in which distal portion has previously been killed due to girdling by *D. septosporum* infection. C: two bands present on live needle (40X). D: four bands present on live needle (10X). E: one band present on dead needle (40X). F: six bands present on dead needle (40X).

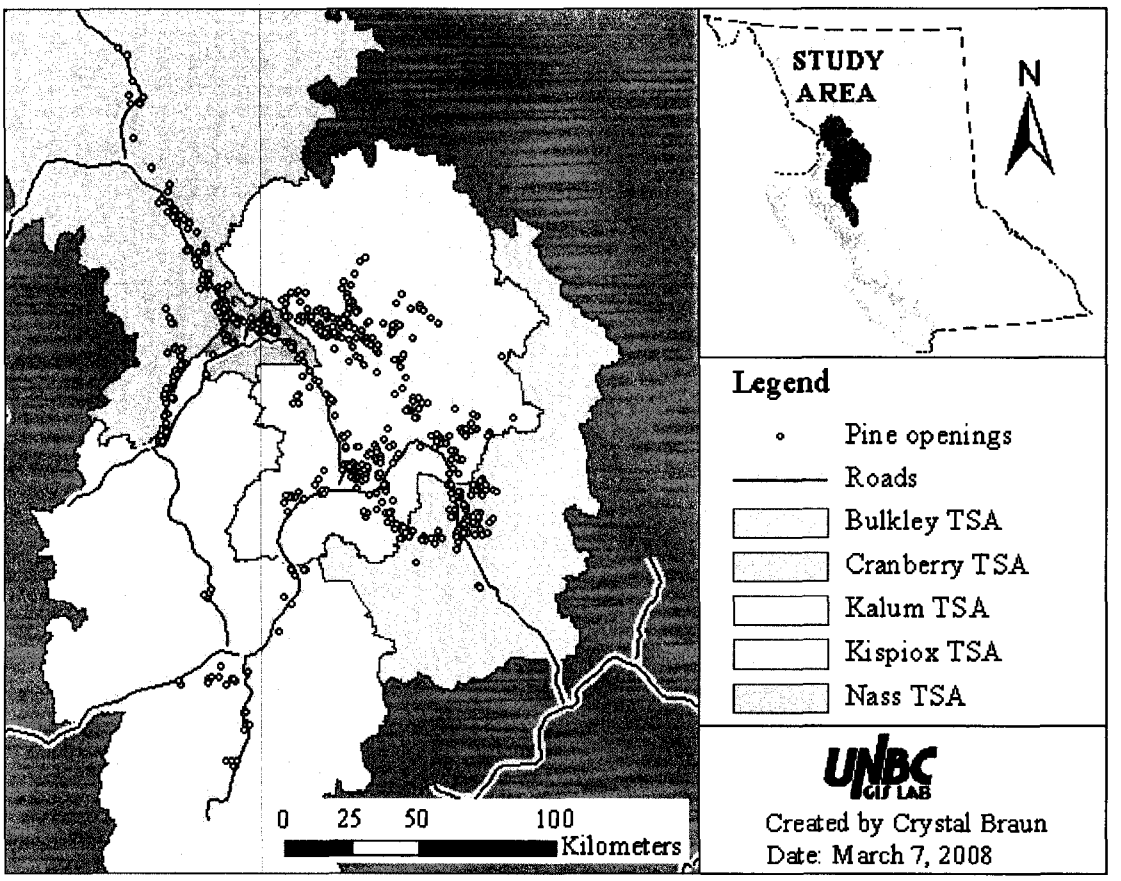

Figure 2.3. Distribution of pine openings in northwestern British Columbia assessed for Dothistroma needle blight in the 2006 aerial survey (BCMOFR 2006).

Table 2.1. Biogeoclimatic zones of areas covered in the 2006 aerial survey for Dothistroma needle blight.

Timber Supply Area	Area surveyed (ha)	Biogeoclimatic zone distribution (%)			
		ICH	CWH	SBS	ESSF
Bulkley	2 821.0	62.4	-	15.1	15.1
Cranberry	2 141.1	100.0	-	-	-
Kalum	1 012.1	33.3	63.2	-	-
Kispiox	8 457.8	96.2	3.5	0.2	-
Nass	3 803.8	100.0	-	-	-
Total Area	18 235.8	86.0	7.7	2.4	2.3

CHAPTER 3: RESULTS

3.1 Plot monitoring and weather analysis results

Severity of disease caused by *Dothistroma septosporum* (Dorog.) Morelet in the study plots ranged from low (100% functional live crown) to high (<5% functional live crown) severity. At the beginning of the study period the majority of selected lodgepole pine (*Pinus contorta* var *latifolia* Dougl. Ex Loud.) showed some level of infection by *D. septosporum*, altogether having an average functional live crown (FLC) of 37.5 ± 31.2% (Figure 3.1). The study period extended from the beginning of June until late September, when disease symptoms were no longer observed on the selected needles.

3.1.1 Red banding analysis

D. septosporum red bands were detected from week 1 to week 16 (Table 3.1), indicating points of successful *D. septosporum* infection and colonization. Their intensity peaked from July to August, fading sooner than fruiting bodies and very gradually decreasing towards the end of the summer (Figure 3.2). By August loss of the red color in *D. septosporum* red bands was observed on heavily infected needles (Figure 3.3). This coincided with the development of *D. septosporum* fruiting bodies on dead needle tissue outside or in the absence of red banding.

The average temperature, relative humidity, and leaf wetness on days which red bands were observed were respectively 12.8 ± 1.9°C, 85.6 ± 8.4%, and 55.4 ± 15.2% (Figure 3.4). Paired two-sample *t*-tests revealed no difference between the mean daily temperature or leaf wetness for red banding and fruiting body development or spore production (see Table 3.2). However the average daily relative humidity was significantly different between red banding and fruiting body production, but not with spore production (see Table 3.2).

Warm temperatures, high humidity, and the presence of moisture appear conducive to the development of red banding (Figures 3.5 and 3.6). In all red banding models, numerous temperature, relative humidity, and leaf wetness thresholds calculated over 4- and 7-day periods correlated with disease development. Although many weather variables were eliminated when tested against each other, for the following models the number of explanatory variables was further reduced in the interest of ecological simplicity. The best models contain the weather variables above several thresholds that were the most consistent between 4- and 7-day potential models (Table 3.3). For definitions of the terms used in the red banding models, see Table 3.4.

The following weather variables were positively correlated with red banding development. Temperatures in 4-day models switched from nightly minimums $\geq 7^{\circ}C$ to daily means $\geq 10^{\circ}C$ in 7-day models. Maximum nightly temperatures $\geq 18^{\circ}C$ were also consistent in all models except the 7-day model with leaf wetness. Relative humidity $\geq 70\%$ and leaf wetness $\geq 40\%$ were consistent variables between 4- and 7-day models. The most parsimonious model would be the 7-day temperature and leaf wetness mixed effects model, as it is consistent with the data collection over weekly intervals. It also uses mean temperature rather than minimum and maximum temperatures in pairs. Models with relative humidity were not considered as relative humidity was tested against leaf wetness variables and was not retained in temperature and leaf wetness models.

3.1.2 Fruiting body analysis

D. septosporum fruiting bodies were detected from week 1 to week 16 (Table 3.1), indicating the amount of pathogen present. Their intensity peaked from July to August, persisting later than observed red bands but gradually decreasing towards the end of the

summer (Figure 3.2). From the start of the study period *D. septosporum* fruiting bodies were easy to identify on the basis of a black fruiting body emerging from beneath a flap of needle epidermis. However fruiting bodies were no longer counted when they lost these identifiable characteristics or were indistinguishable from emerging saprotrophic fungi (Figure 3.7).

The average temperature, relative humidity, and leaf wetness on days which fruiting bodies were observed were respectively 12.9 ± 1.9°C, 87.1 ± 7.2%, and 57.0 ± 14.4% (Figure 3.4). These variables did not differ statistically from periods when fruiting bodies were not observed, as was indicated by two-sample *t*-tests (see Table 3.5). They also did not differ significantly from mean daily temperature, relative humidity, or leaf wetness during periods when spores were produced (see Table 3.2).

As with red bands, warm temperatures and high humidity appeared to be conducive to *D. septosporum* fruiting body development (Figure 3.8). The presence of moisture also appeared to play an important role in fruiting body emergence (Figure 3.9). Fruiting bodies were never observed on days when needles had been completely dry (when average leaf wetness was < 30%). The adaxial surface of needle pairs in which water could be retained was commonly observed to have dense fruiting body emergence. However high numbers of fruiting bodies were not restricted to this area.

Many temperature, relative humidity, and leaf wetness thresholds correlated with disease development in all fruiting body models. However, in the interest of ecological simplicity the number of explanatory variables was reduced for the following models. The best models contained the most consistent weather variables over 4- and 7-day periods. 4- and 7-day mixed effects models described in Table 3.6 hold for 10-25 year old pine in the

Skeena Stikine and Kalum Forest Districts. For definitions of the terms used in the fruiting body models, see Table 3.7.

All weather variables described were positively correlated with fruiting body development, with the exception of daily leaf wetness ≥ 90% in a 4-day model. A nightly mean temperature variable was the only variable retained in the 4-day temperature and relatively humidity model. Temperature variables in the rest of the models all referred to daily temperatures, ranging from a minimum ≥ 6°C to a mean ≥ 7°C. These levels were consistent with the nightly temperature requirements for red banding development, which ranged from a minimum ≥ 7°C to a mean ≥ 10°C. The presence of leaf moisture (leaf wetness ≥ 30%) was consistent between 4- and 7-day models, as well as with red banding models. Relative humidity was higher in fruiting body models compared to red banding models. Maximum daily relative humidity levels ≥ 90% were retained in fruiting body models, compared to a minimum ≥ 70% in red banding models. Relative humidity was only retained in the 7-day temperature and relative humidity fruiting body model.

The most parsimonious fruiting body development model is the 7-day temperature and leaf wetness mixed effects model, as it uses single temperature and leaf wetness variables and is consistent with the data collection over weekly intervals.

3.1.3 Spore production analysis

D. septosporum conidia with observed dimensions of 18-35 x 3 μm were captured 5.9% of the time using the needle dip method. Conidia were detected from week 4 to week 13 (Table 3.1), and their production peaked in late July when red banding was starting to decline and prior to the late summer peak in fruiting body development (Figure 3.10). The average temperature, relative humidity, and leaf wetness on days which conidia were

detected were 13.3 ± 1.8°C, 85.7 ± 5.3%, and 58.3 ± 12.4% respectively (Figure 3.4). There were significant differences between periods in which conidia were and were not detected for mean daily temperature, relative humidity, and leaf wetness (see Table 3.5). However spore production analysis could not be completed, as there were not enough data. For a summary of how minimum nightly temperatures and mean daily leaf wetness – two important explanatory variables identified in red banding and fruiting body development – compared between periods in which conidia were and not detected, see Figure 3.11.

3.1.4 Frequency of the sexual stage

Ascospores were never detected by means of the needle dip method. Therefore, the timing of ascospore production could not be determined. From 2670 fruiting body dissections, ascomata were detected twice in samples taken Aug 9, 2007 and Sept 16, 2007 from the Helen Lake site. The reproductive stage was confirmed by the presence of ascospores on the fruiting body (Figure 3.12). No analysis was performed due to insufficient data.

3.2 Topography analysis results

The 608 pine-leading plantations in the 2006 aerial survey showed 40.9 ± 20.7% FLC (Figure 3.1). These plantations had a mean elevation of 491.2 ± 230.3 m, a mean slope of 13.3 ± 10.4%, and a mean distance to the nearest water body of 206.8 ± 170.4 m (Table 3.8). Logistic regression models outlined in Table 3.9 hold for areas with an elevation of 46-1268 m, easting of 473515.6-619578.8, and northing of 6000533-6105250. For definitions of the terms used in the following models, see Table 3.10. No spatial autocorrelation was detected in the models using the northings and eastings quadratic terms.

Using a FLC threshold of 5% to distinguish pine-leading stands likely to recover from those unlikely to recover, differences were observed for elevation (Figure 3.13) and aspect (Figure 3.14). No differences were observed for slope (Figure 3.15) or distance to the nearest water body (Figure 3.16). At a FLC threshold of 10%, differences were also observed in elevation (Figure 3.17) and aspect (Figure 3.18) between stands likely and unlikely to recover. Only differences in elevation were observed for FLC thresholds of 20% (Figure 3.19) and 50% (Figure 3.20).

Elevation had the largest coefficient in the y_{10} logistic regression model. Aspect had larger coefficients in the y_5 model than the y_{10} model. The y_{20} model is the most concise model as it only utilizes elevation, eastings, and northings. However greater sensitivity could be attained in the y_{10} model because it also incorporates aspect.

3.2.1 Disease hazard rating system

Dothistroma Needle Blight Hazard Rating Approach

Low	Elevation > 510 m South-facing aspects
Intermediate	Elevation 235 – 510 m East-, west-, and north-facing aspects
High	Elevation < 235 m East-, west-, and north-facing aspects

Upper and lower elevation limits were determined from the average elevations over which Dothistroma-infected pine plantations were distributed, as defined by the 10% and 20% FLC thresholds (Figures 3.17 and 3.19). Elevation means for pine plantations likely and unlikely to recover were averaged between the FLC thresholds and rounded down for a conservative estimate.

3.2.2 Risk prediction maps

Maps predicting substantial needle loss for 10-25 year old pine plantations with given topographic variables in a Dothistroma needle blight epidemic were generated using the y_{10} and y_{20} logistic regression models (see Table 3.9). They were produced in 25 x 25 m GRID format as single precision coverages (32-bit floating point). As shown in Figures 3.21 and 3.22, graphical results for the y_{10} and y_{20} models were similar. Both displayed road and river corridors as high risk areas for severe Dothistroma infection in the Cranberry Timber Supply Area.

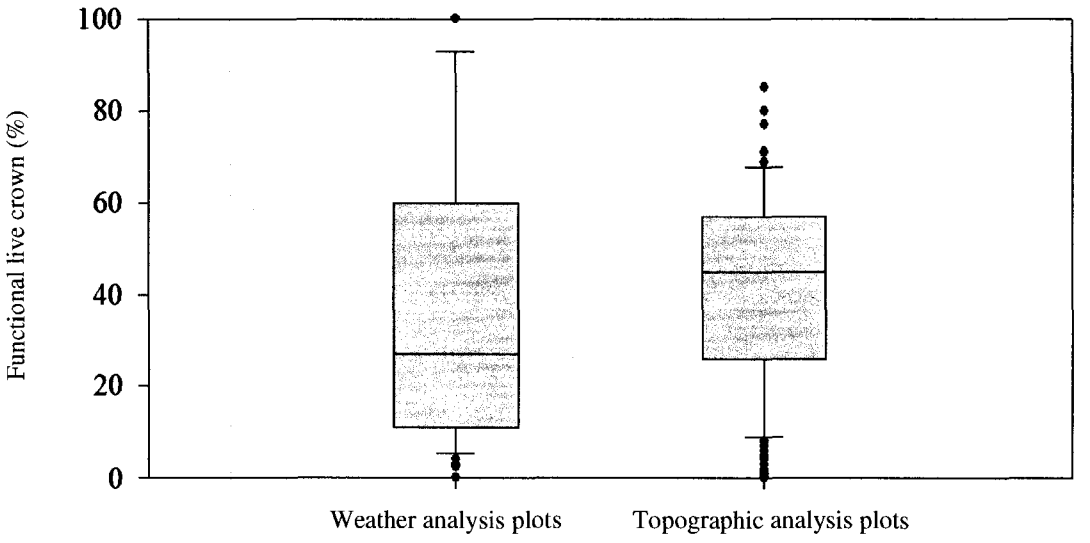

Figure 3.1. Disease severity, as a measure of functional live crown, caused by *D. septosporum* on lodgepole pine trees of the Skeena Stikine Forest District (weather analysis plots) and pine-leading plantations in the 2006 aerial survey of the Skeena Stikine and Kalum Forest Regions (topographic analysis plots). The box spans the interquartile range (IQR) from the 25th to 75th percentile, the bar identifies the median, and the whiskers extend to 1.5 times the IQR.

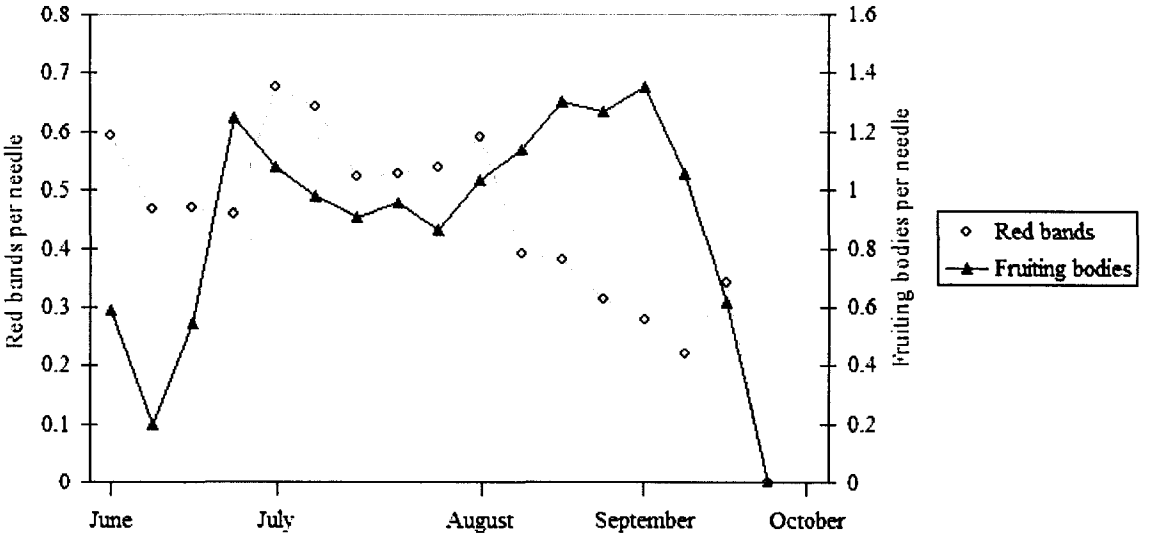

Figure 3.2. Average number of red bands and fruiting bodies on lodgepole pine needles of the Skeena Stikine Forest District study plots over the 2007 field season.

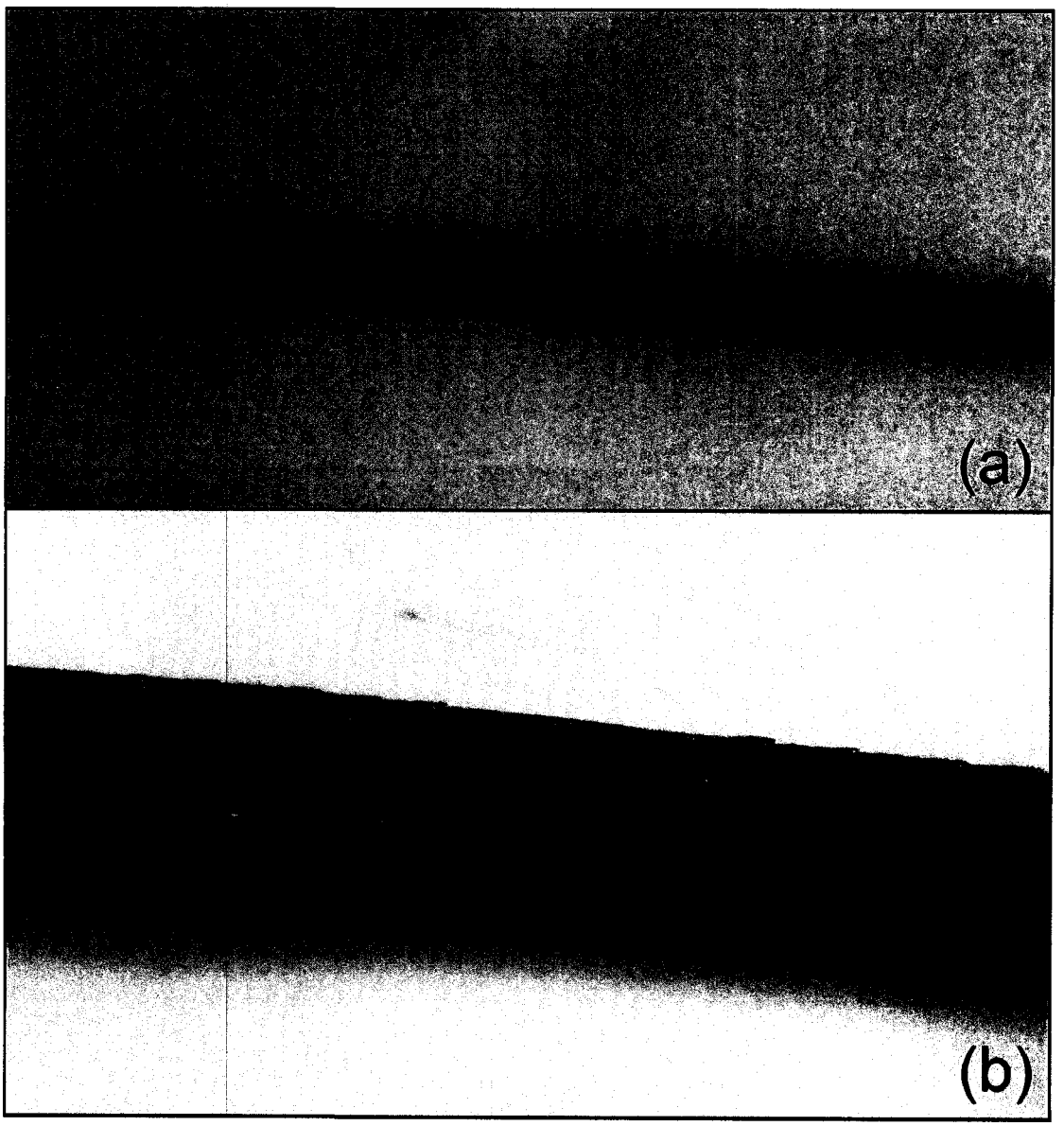

Figure 3.3. Development of *D. septosporum* fruiting bodies in the absence of red banding on lodgepole pine needle tissue. A: 10X magnification. B: 40X magnification.

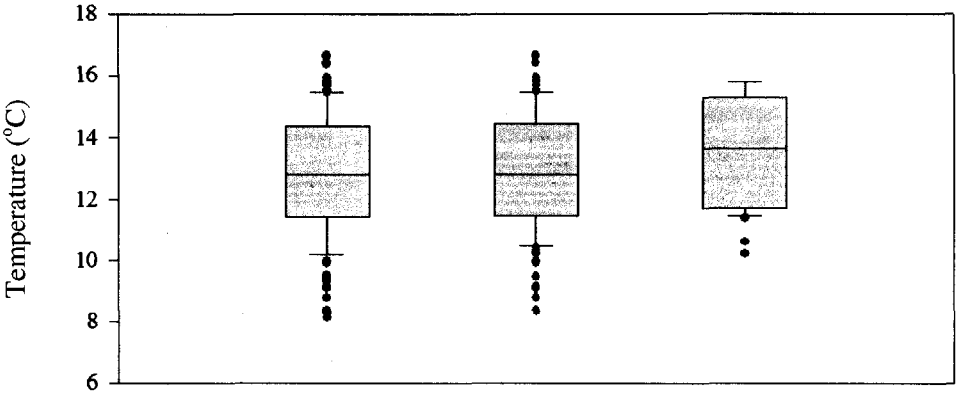

Figure 3.4a. Average daily temperature for *D. septosporum* symptoms.

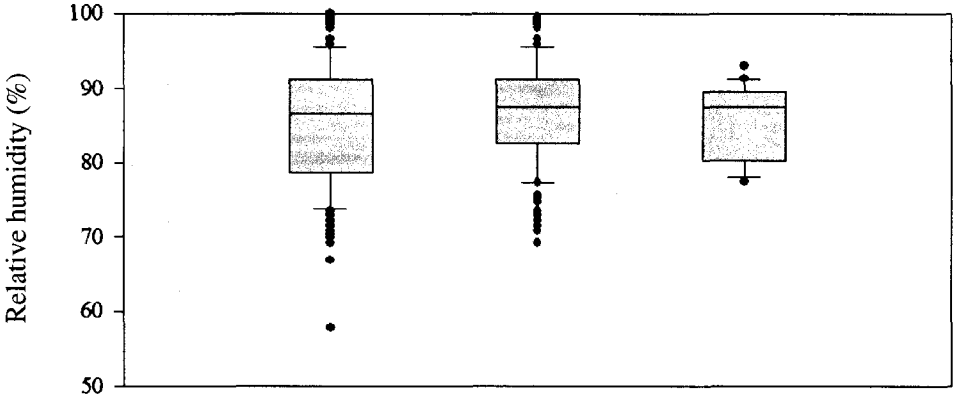

Figure 3.4b. Average daily relative humidity for *D. septosporum* symptoms.

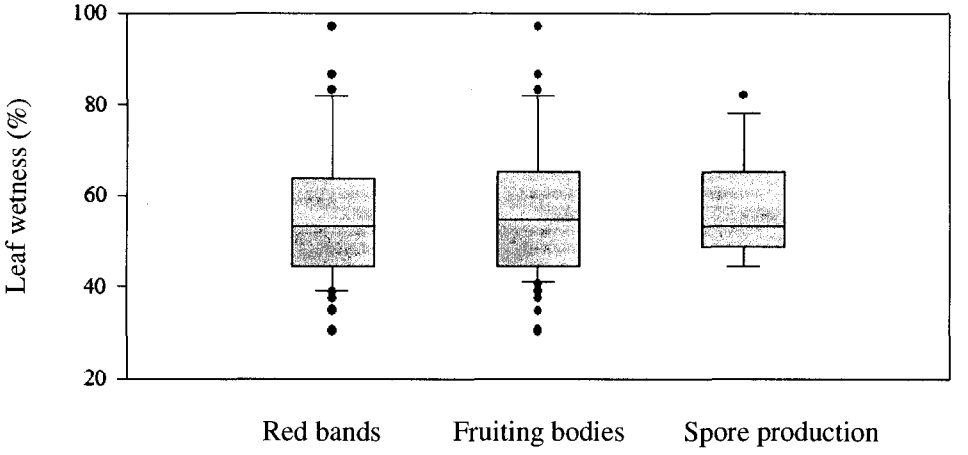

Figure 3.4c. Average daily leaf wetness for *D. septosporum* symptoms.

Figure 3.4. Average daily temperature (a), relatively humidity (b), and leaf wetness (c) when *D. septosporum* symptoms were observed on lodgepole pine needles in the Skeena Stikine Forest District study plots. The box spans the IQR from the 25th to 75th percentile, the bar identifies the median, and the whiskers extend to 1.5 times the IQR.

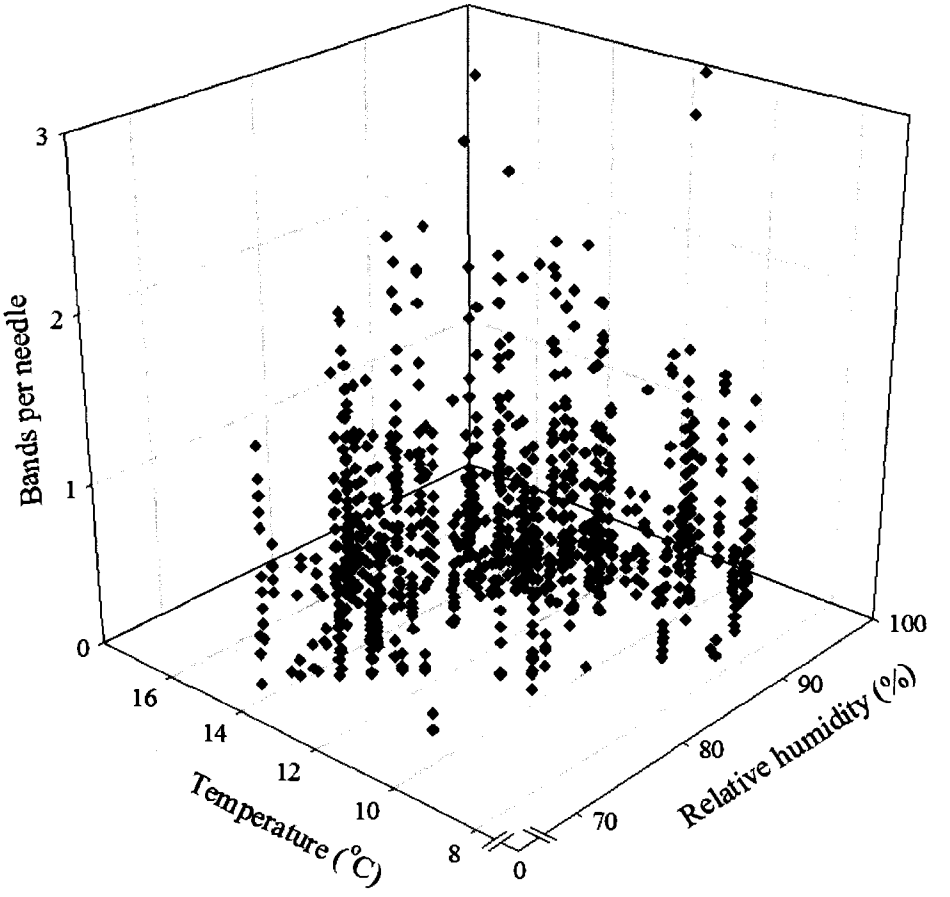

Figure 3.5. Plot of average daily temperature vs average relative humidity vs the number of red bands on pine needles.

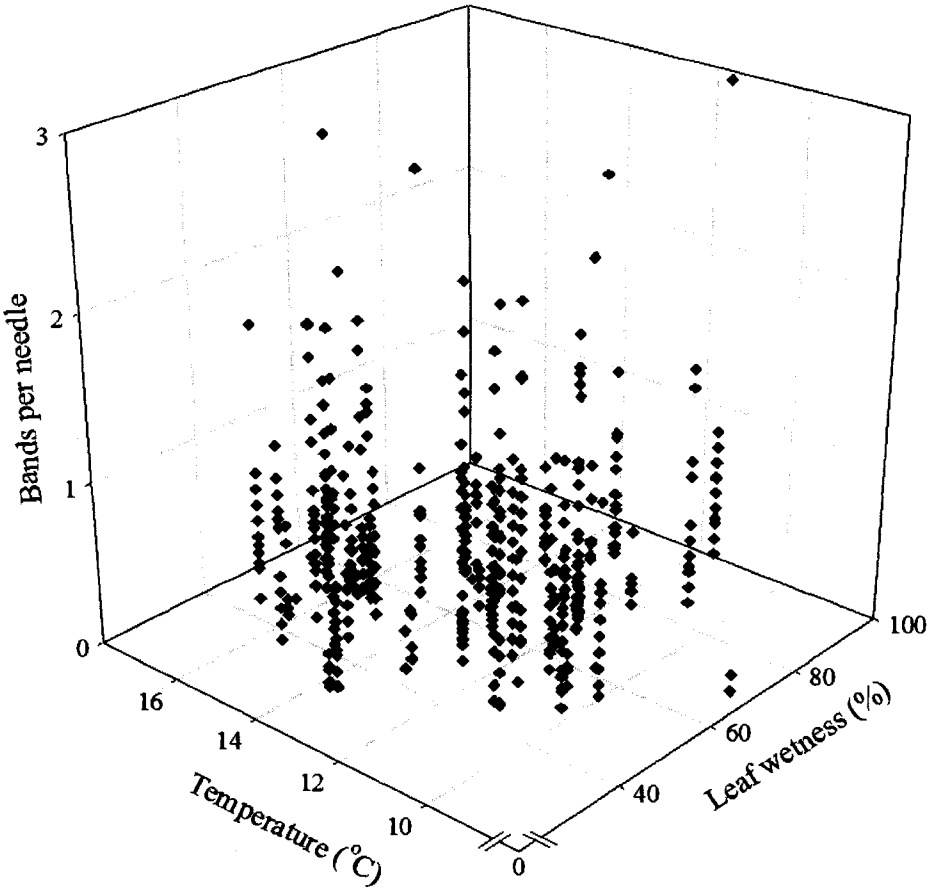

Figure 3.6. Plot of average daily temperature vs average daily leaf wetness vs the number of red bands on pine needles.

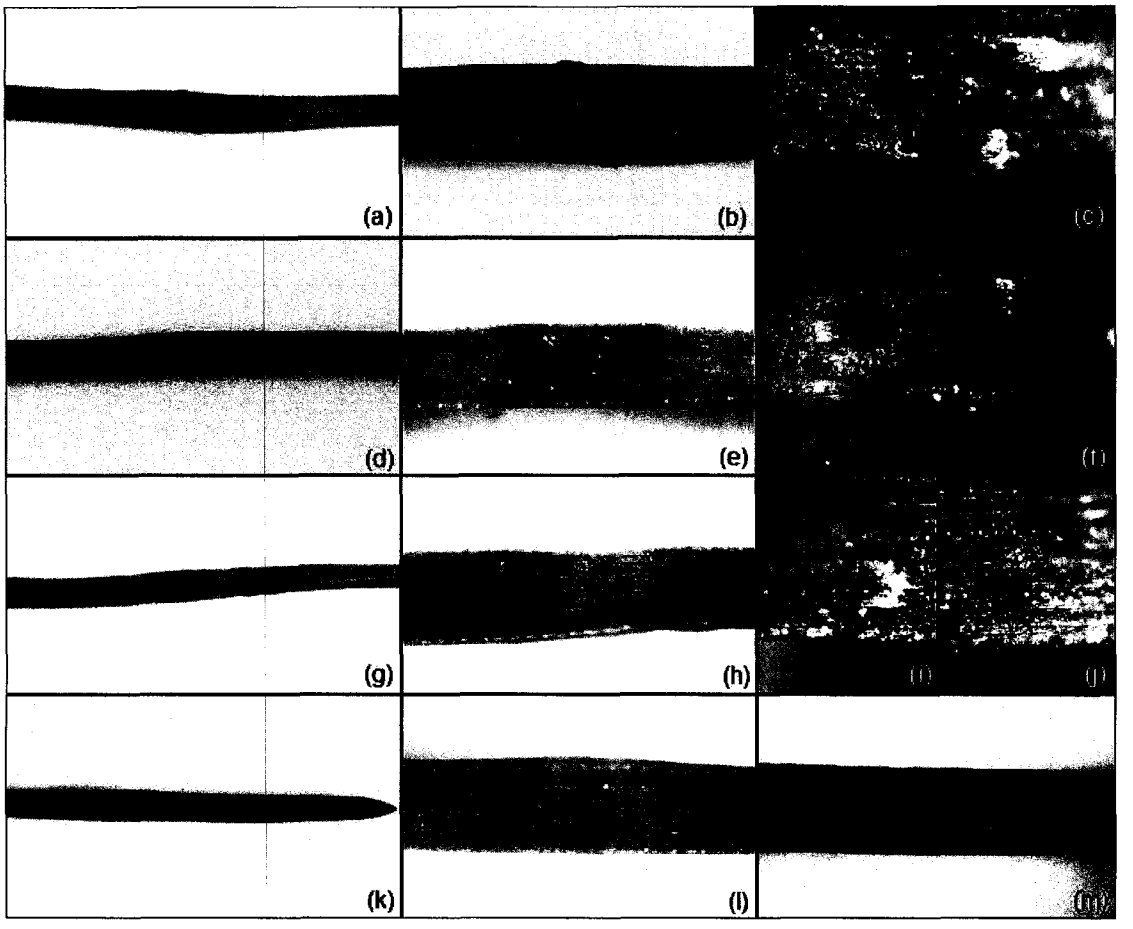

Figure 3.7. Variation in the appearance of fruiting bodies on pine needles. A-C: *D. septosporum* fruiting bodies (A: 10X, B: 40X, C: 100X). D-F: *D. septosporum* fruiting bodies on red bands with unidentified fruiting bodies developing in adjacent tissues (D: 10X, E: 40X, F: 100X). Only the fruiting bodies on the red band with epidermal flaps would have been counted. G-J: Older *D. septosporum* fruiting bodies on senesced pine needle (G: 10X, H: 40X, I: 100X, J: 100X). I: Close-up of left fruiting body seen in (G); would have been counted as it retains an epidermal flap visible. J: Close-up of right fruiting body seen in (G); would not have been counted. K: Old *D. septosporum* fruiting bodies on senesced pine needle (10X). L: Close-up of left side of needle (40X), showing *D. septosporum* fruiting bodies which no longer raise the epidermal flaps. M: Close-up of right side of needle (40X), showing *D. septosporum* fruiting bodies which have lost their epidermal flaps. The fruiting bodies in K and L would be excluded from the observations.

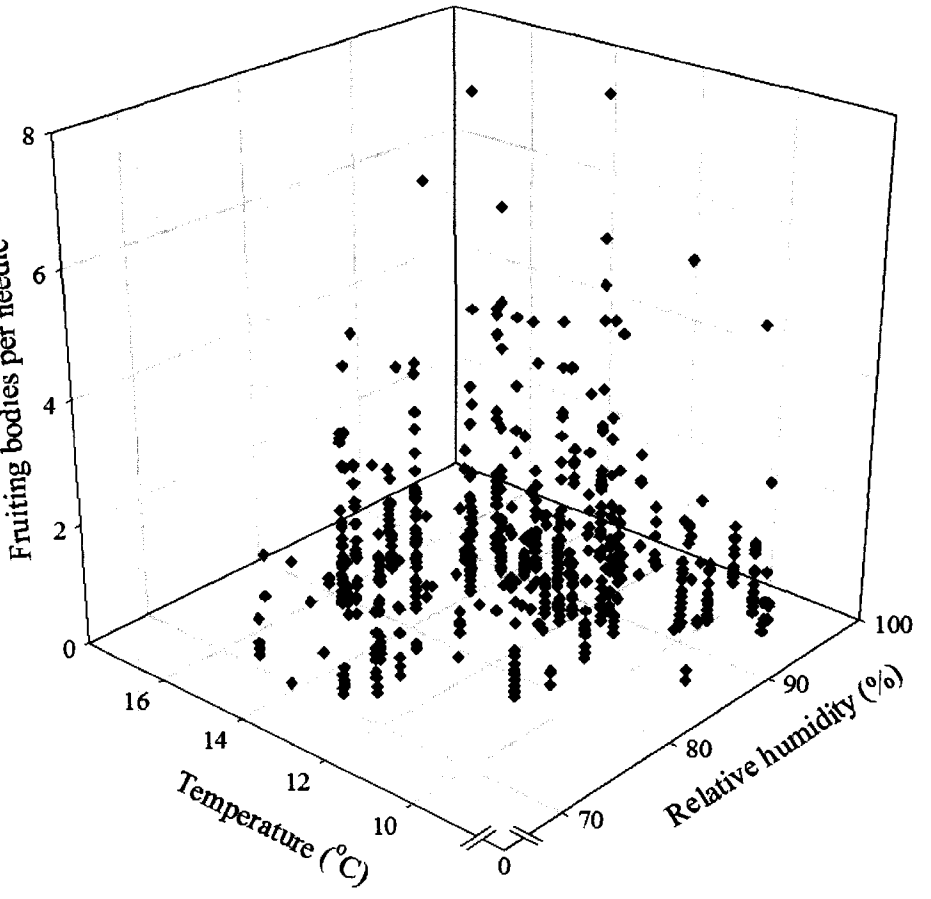

Figure 3.8. Plot of average daily temperature vs average daily relative humidity vs the number of fruiting bodies on pine needles.

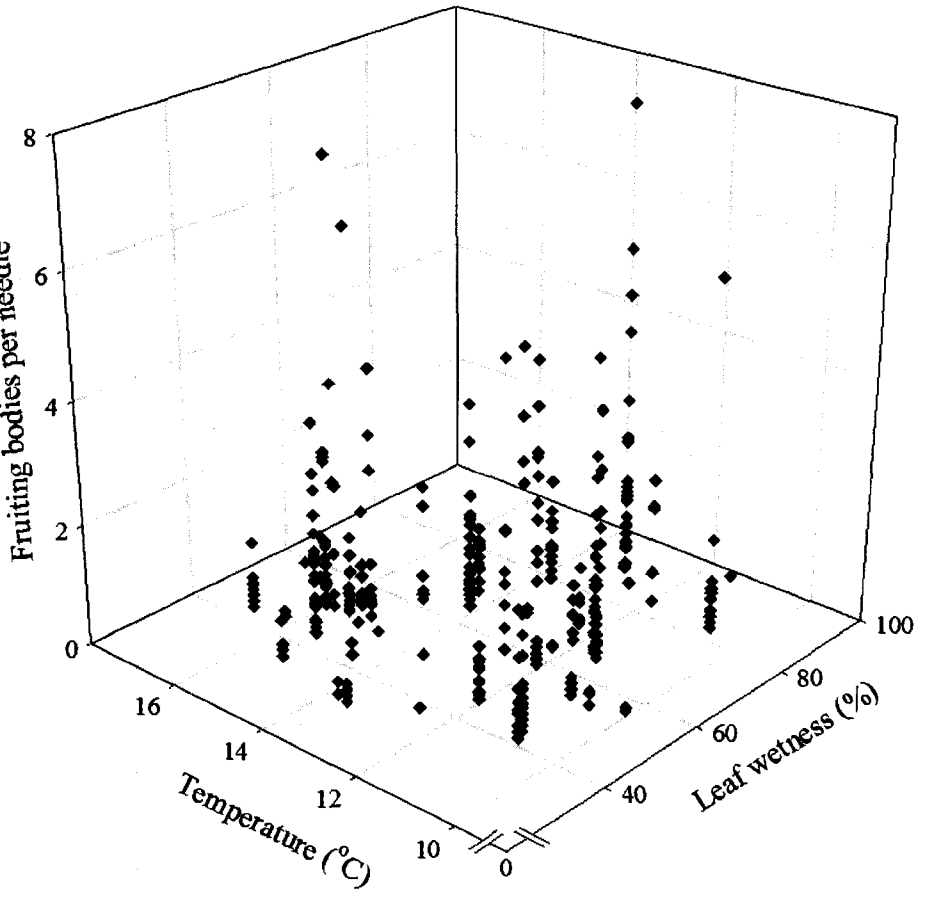

Figure 3.9. Plot of average daily temperature vs average daily leaf wetness vs the number of fruiting bodies on pine needles.

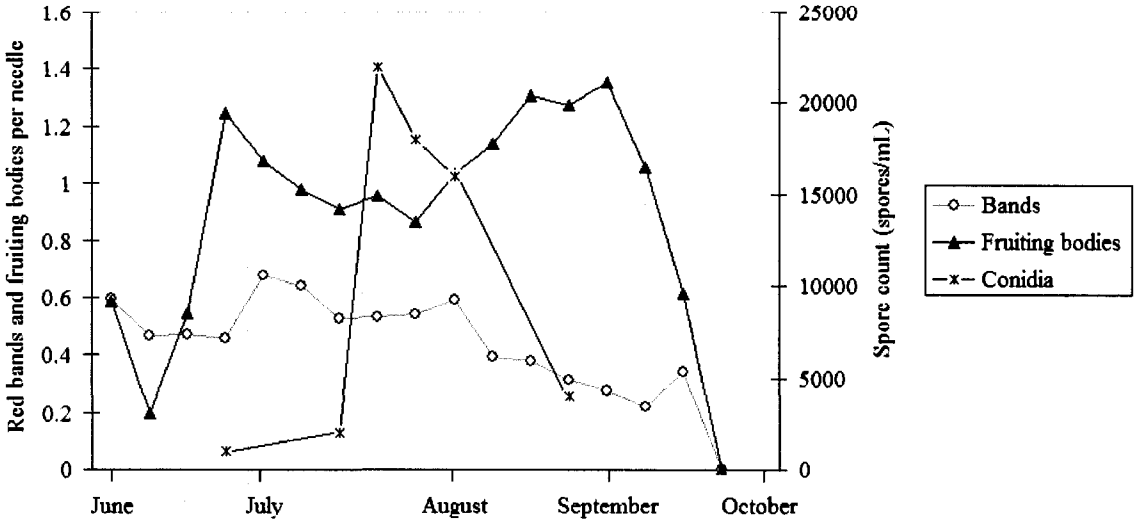

Figure 3.10. *D. septosporum* symptom development on lodgepole pine needles of the Skeena Stikine Forest District study plots over the 2007 field season.

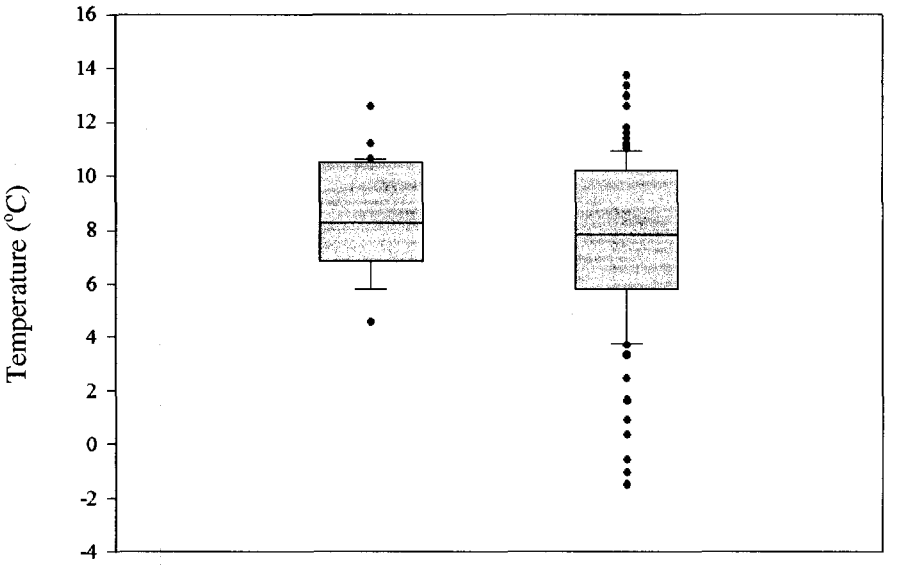

Figure 3.11a. Minimum nightly temperature for periods when conidia were and were not detected.

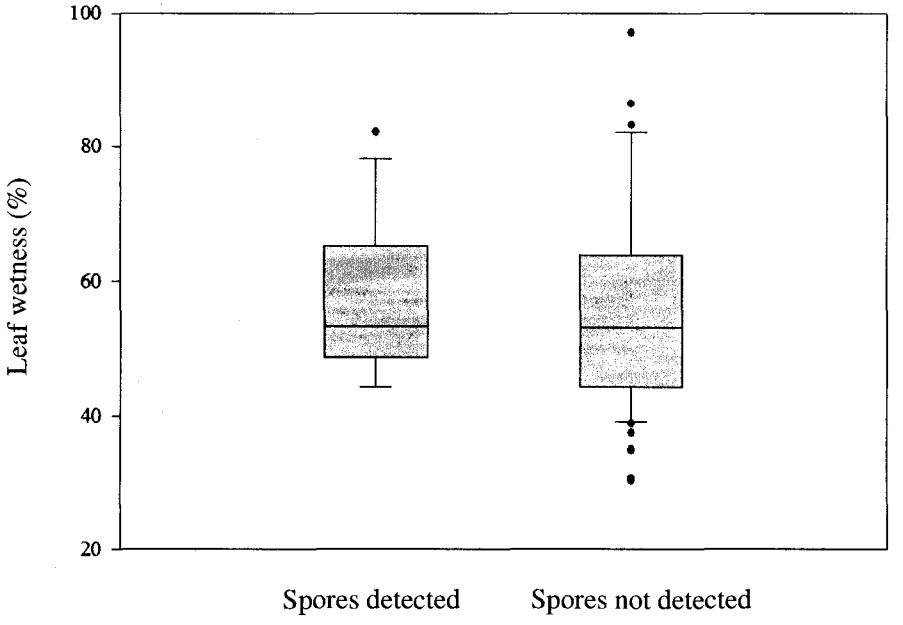

Figure 3.11b. Average daily leaf wetness for periods when conidia were and were not detected.

Figure 3.11. Minimum nightly temperature (a) and daily leaf wetness (b) periods when spores were and were not detected on lodgepole pine needles in the Skeena Stikine Forest District study plots. The box spans the IQR from the 25th to 75th percentile, the bar identifies the median, and the whiskers extend to 1.5 times the IQR.

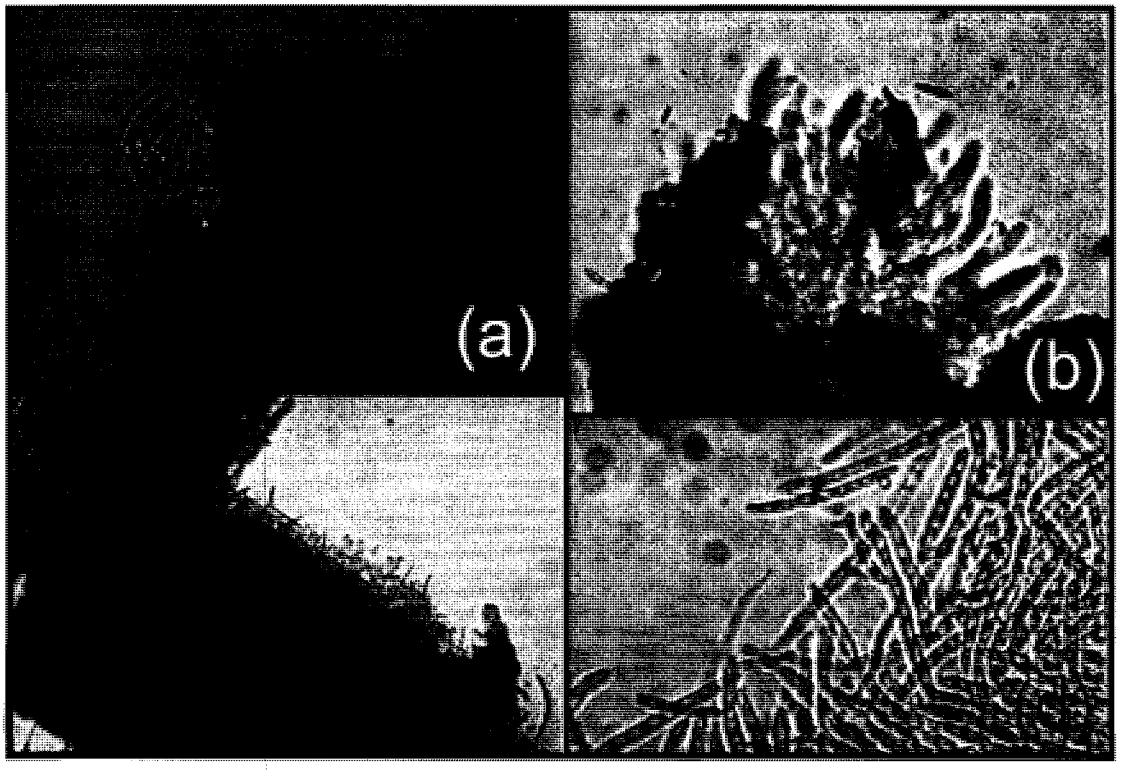

Figure 3.12. The reproductive stages of *D. septosporum*. A: Ascomata bearing ascospores (100X). B: Ascospores (400X). C: Stromata bearing conidia (100X). D: Conidia (400X).

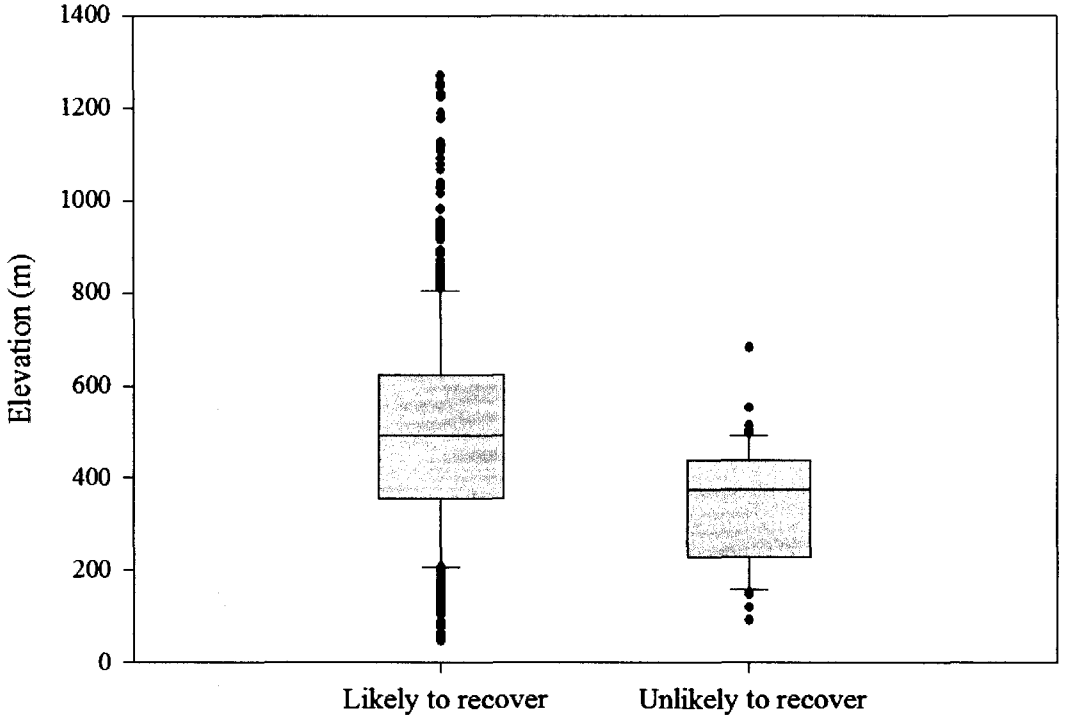

Figure 3.13. Elevation of Dothistroma-infected openings by recovery likelihood using a functional live crown threshold of 5%. The box spans the IQR from the 25th to 75th percentile, the bar identifies the median, and the whiskers extend to 1.5 times the IQR.

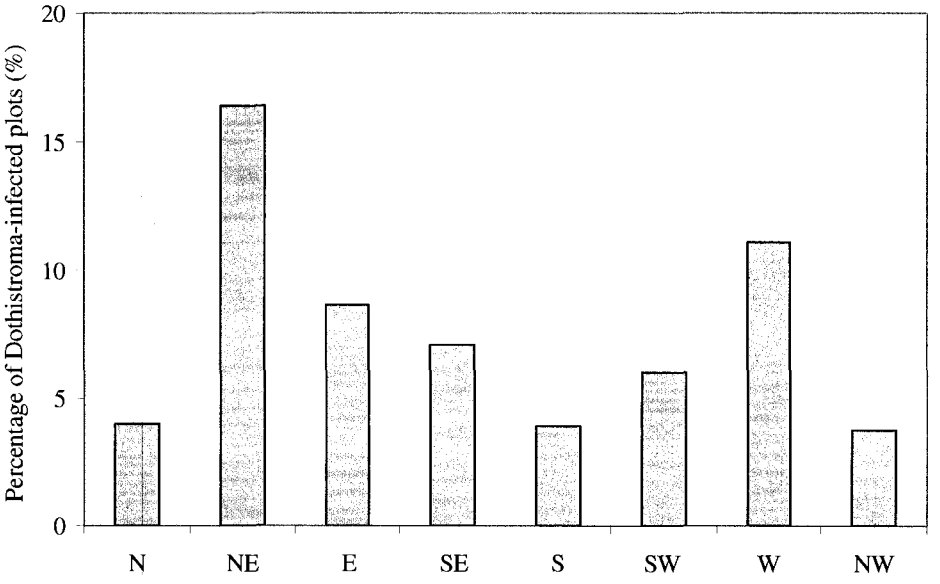

Figure 3.14. Aspect of Dothistroma-infected openings considered unlikely to recover using a functional live crown threshold of 5%.

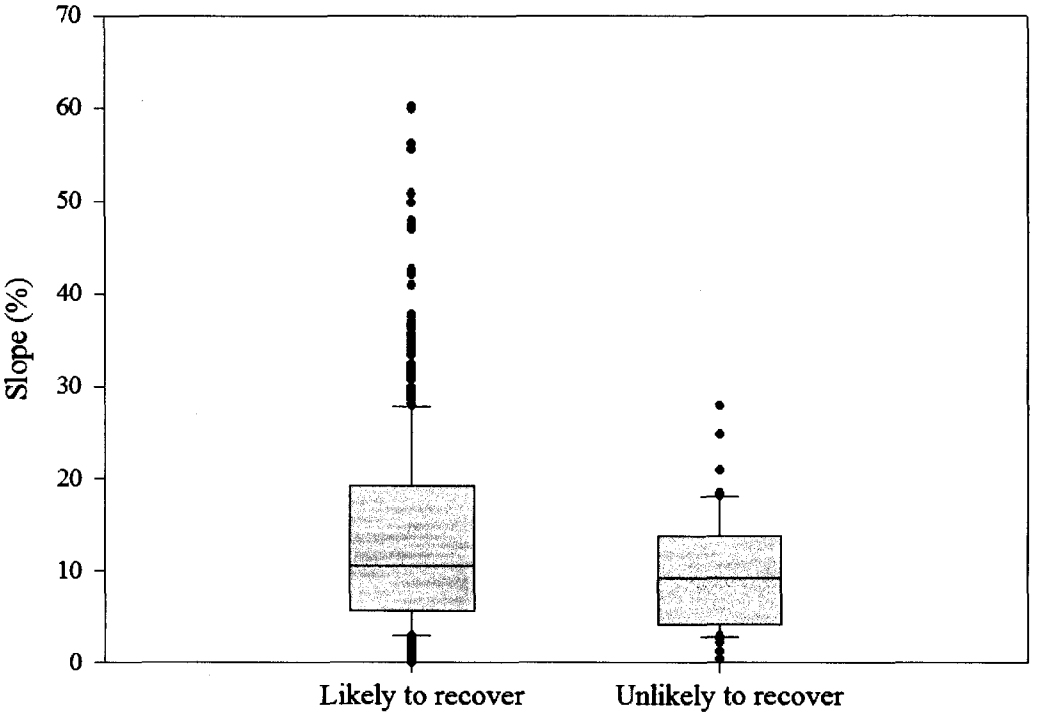

Figure 3.15. Slope of Dothistroma-infected openings by recovery likelihood using a functional live crown threshold of 5%. The box spans the IQR from the 25th to 75th percentile, the bar identifies the median, and the whiskers extend to 1.5 times the IQR.

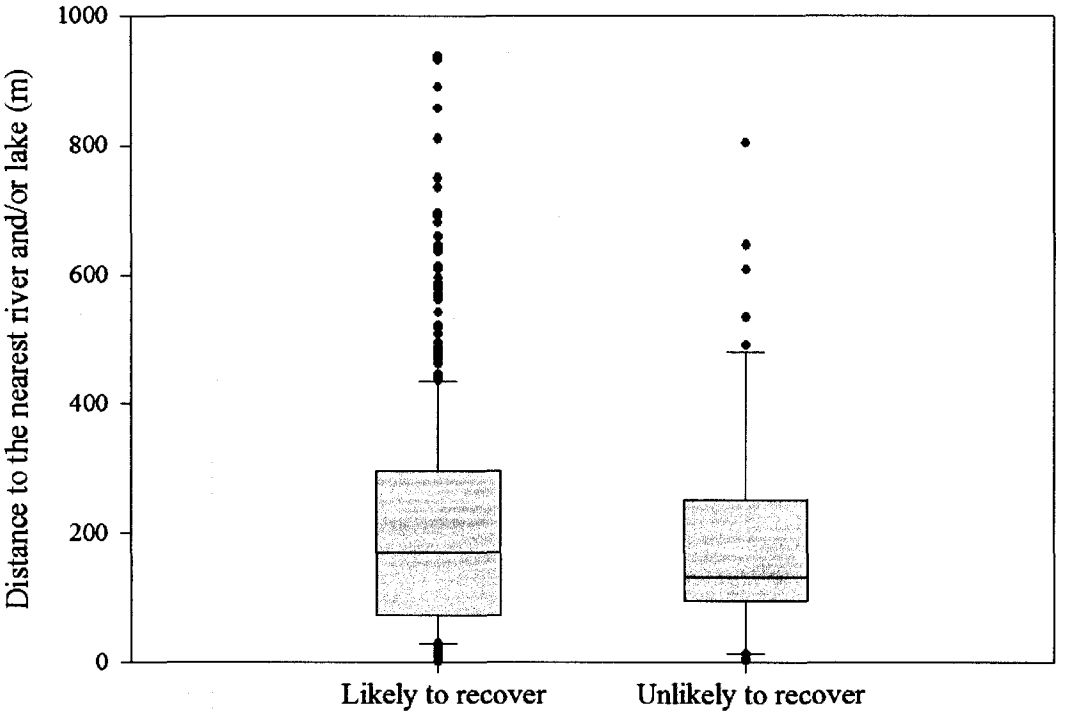

Figure 3.16. Distance of Dothistroma-infected openings to the nearest river and/or lake by recovery likelihood using a functional live crown threshold of 5%. The box spans the IQR from the 25th to 75th percentile, the bar identifies the median, and the whiskers extend to 1.5 times the IQR.

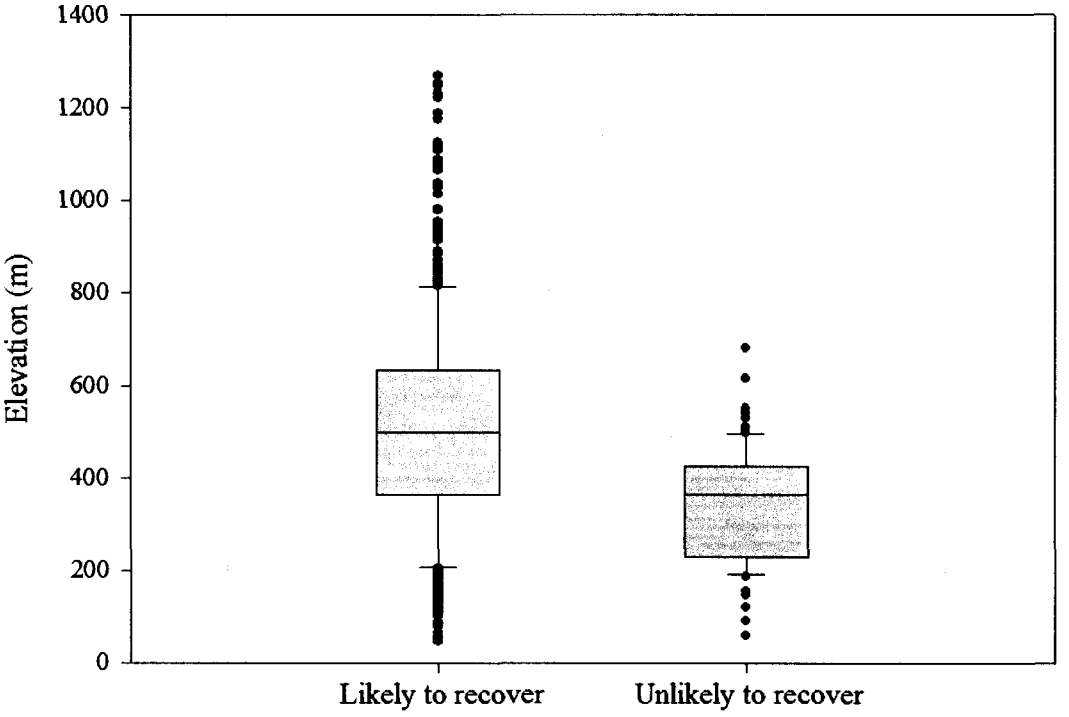

Figure 3.17. Elevation of Dothistroma-infected openings by recovery likelihood using a functional live crown threshold of 10%. The box spans the IQR from the 25th to 75th percentile, the bar identifies the median, and the whiskers extend to 1.5 times the IQR.

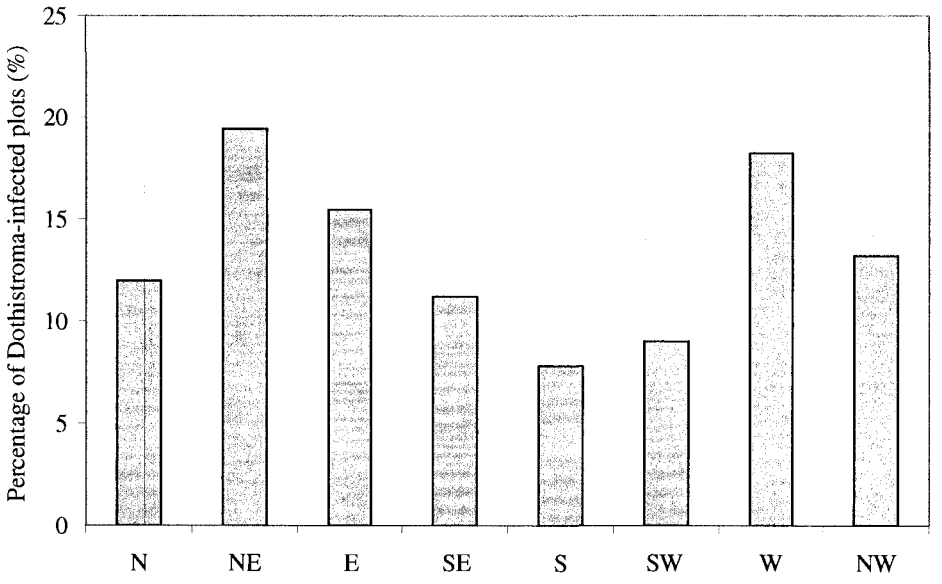

Figure 3.18. Aspect of Dothistroma-infected openings considered unlikely to recover using a functional live crown threshold of 10%.

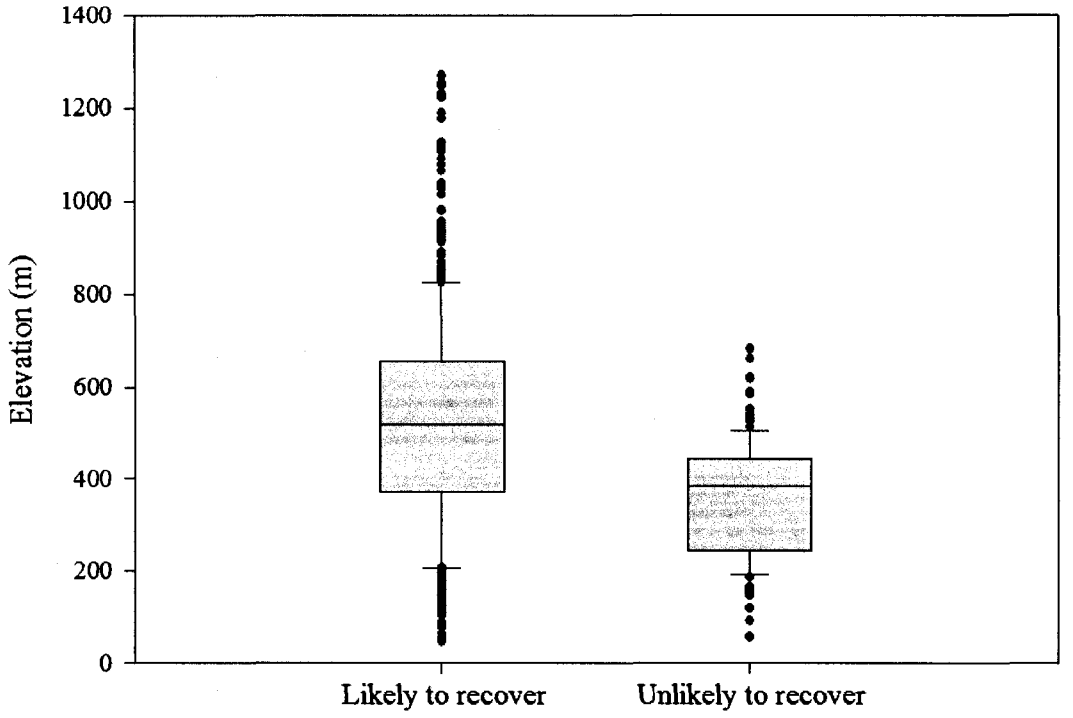

Figure 3.19. Elevation of Dothistroma-infected openings by recovery likelihood using a functional live crown threshold of 20%. The box spans the IQR from the 25th to 75th percentile, the bar identifies the median, and the whiskers extend to 1.5 times the IQR.

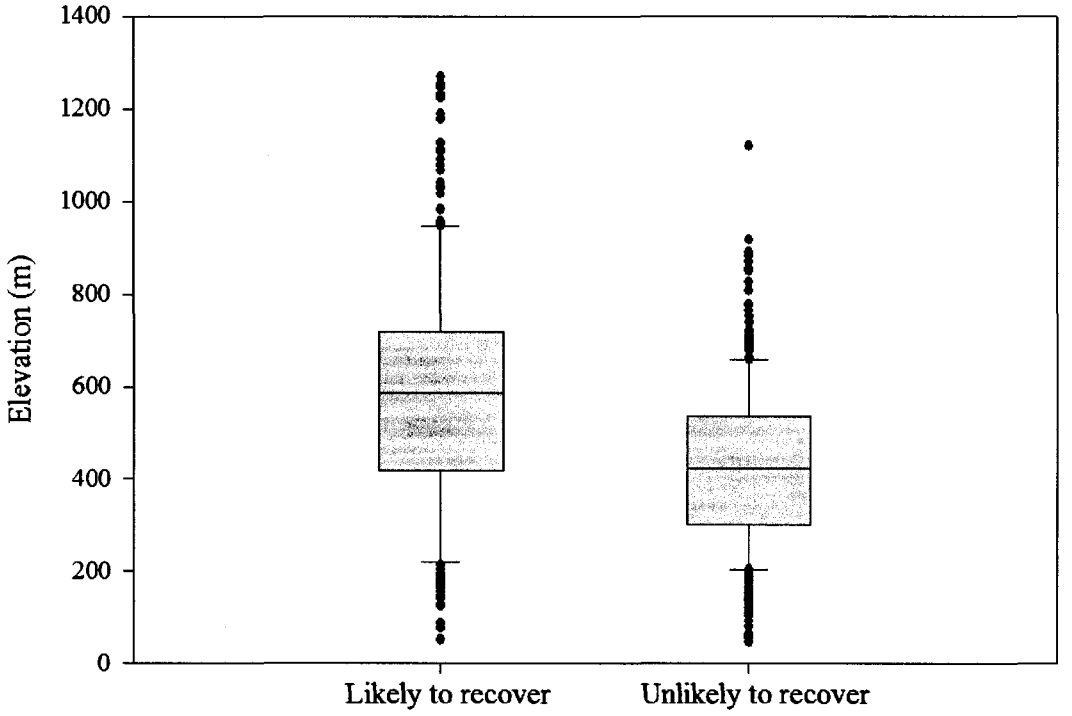

Figure 3.20. Elevation of Dothistroma-infected openings by recovery likelihood using a functional live crown threshold of 50%. The box spans the IQR from the 25th to 75th percentile, the bar identifies the median, and the whiskers extend to 1.5 times the IQR.

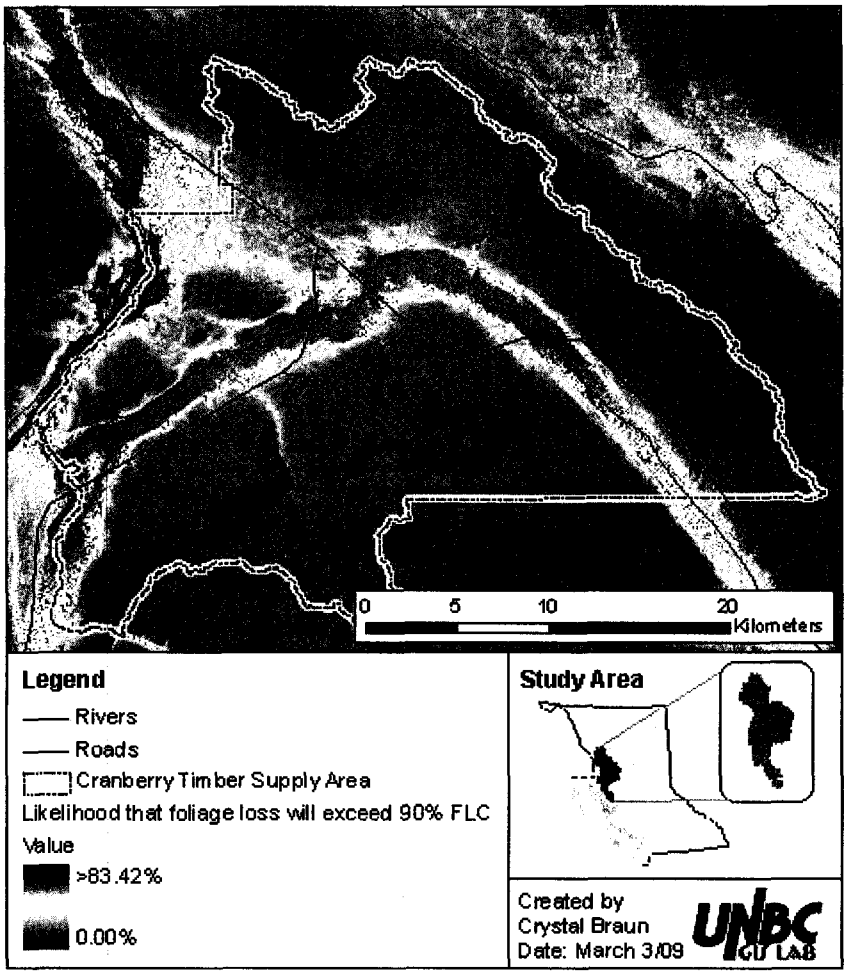

Figure 3.21. Risk prediction map for substantial needle loss or death in lodgepole pine from Dothistroma needle blight infection in the Cranberry TSA. The prediction surface created from the backtransformation of the logistic regression model using a 10% FLC threshold. As shown in the Study Area mini-map, the Cranberry TSA represents a small fraction of the total area represented by the five TSAs covered in the 2006 aerial survey.

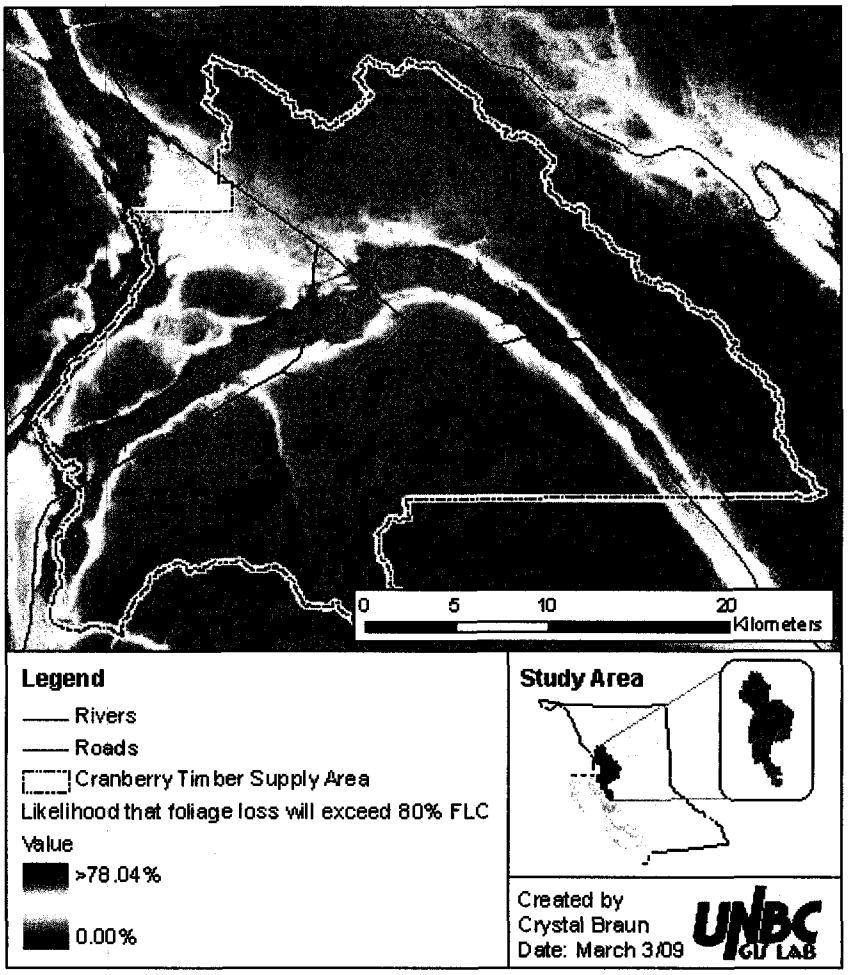

Figure 3.22. Risk prediction map for growth loss in lodgepole pine from Dothistroma needle blight infection in the Cranberry TSA. The prediction surface created from the backtransformation of the logistic regression model using a 20% FLC threshold. As shown in the Study Area mini-map, the Cranberry TSA represents a small fraction of the total area represented by the five TSAs covered in the 2006 aerial survey.

Table 3.1. Onset dates for observed *D. septosporum* symptoms by plot from the 2007 field season.

Site	Plot	Red banding		Fruiting body production		Spore production	
		Week	Date	Week	Date	Week	Date
Bulkley	1	9	Jul 31	-	-	-	-
	2	1 - 16	Jun 7 - Sep 16	1 - 15	Jun 7 - Sep 9	4 - 10	Jun 27 - Aug 10
	3	15 - 16	Sep 9 - Sep 16	15	Sep 9	-	-
Cranberry	1	1 - 12	Jun 4 - Aug 19	4 - 10	Jun 24 - Aug 8	-	-
	2	1 - 14	Jun 4 - Sep 3	8 - 14	Jul 24 - Sep 3	-	-
	3	2 - 15	Jun 14 - Sep 8	5 - 14	Jul 4 - Sep 3	8	Jul 25
Helen Lake	1	1 - 16	Jun 5 - Sep 16	1 - 16	Jun 5 - Sep 16	9	Jul 31
	2	1 - 16	Jun 5 - Sep 16	1 - 16	Jun 5 - Sep 16	7 - 10	Jul 19 - Aug 9
	3	1 - 14	Jun 5 - Sep 3	5 - 14	Jul 5 - Sep 3	10 - 13	Aug 9 - Aug 28
Muldoe	1	1 - 15	Jun 6 - Sep 9	2 - 14	Jun 16 - Sep 4	8 - 13	Jul 25 - Aug 29
	2	1 - 15	Jun 6 - Sep 9	3 - 14	Jun 21 - Sep 4	9 - 10	Jul 30 - Aug 9
	3	1 - 8	Jun 6 - Jul 25	6 - 7	Jul 10 - Jul 20	-	-

Table 3.2. Paired two-sample independent *t*-tests comparing daily mean weather variables for days when different *D. septosporum* signs and symptoms were present.

Symptom	Mean daily weather variable	Red banding development			Fruiting body development		
		Test statistic	df	p-value	Test statistic	df	p-value
Fruiting body development	Temperature (°C)	-1.20	2015	0.2290	-	-	-
	Relative humidity (%)	-4.10	2015	**4.343×10^{-5}**	-	-	-
	Leaf wetness (%)	-1.59	999	0.1130	-	-	-
Spore production	Temperature (°C)	-1.79	1349	0.0739	-1.46	742	0.1459
	Relative humidity (%)	-0.098	1349	0.9222	1.19	742	0.2349
	Leaf wetness (%)	-0.966	665	0.3345	-0.46	384	0.6440

Table 3.3. Mixed effect models for *D. septosporum* red banding development.

Model	Variable	Coefficient	SE	Test statistic	p-value	AIC
4 day temperature and relative humidity				t_{1127}	< 0.0001	-200
	Intercept	1.140	0.1032	11.06	< 0.0001	
	DRH70_min4	0.033	0.0090	3.71	< 0.001	
	NT7_min4	0.029	0.0056	5.17	< 0.0001	
	NT18_max4	0.031	0.0057	5.48	< 0.0001	
	NEEDLE	-0.021	0.0064	-3.36	< 0.001	
	TIME	-0.003	0.0002	-11.88	< 0.0001	
4 day temperature and leaf wetness				t_{556}	< 0.0001	-89.3
	Intercept	1.359	0.1454	9.34	< 0.0001	
	DW40_mean4	0.055	0.0123	4.46	< 0.0001	
	NT7_min4	0.027	0.0071	3.79	< 0.001	
	NT18_max4	0.039	0.0076	5.16	< 0.0001	
	NEEDLE	-0.029	0.0098	-2.97	< 0.005	
	TIME	-0.004	0.0004	-10.36	< 0.0001	
7 day temperature and relative humidity				t_{1032}	< 0.0001	-188
	Intercept	1.193	0.1090	10.94	< 0.0001	
	DRH70_min4	0.017	0.0070	2.50	< 0.05	
	NT10_mean7	0.031	0.0073	4.22	< 0.0001	
	NT18_max7	0.023	0.0051	4.41	< 0.0001	
	NEEDLE	-0.027	0.0065	-4.10	< 0.0001	
	TIME	-0.003	0.0003	-12.23	< 0.0001	
7 day temperature and leaf wetness				t_{519}	< 0.0001	-73.7
	Intercept	1.442	0.1583	9.11	< 0.0001	
	DW40_mean7	0.019	0.0079	2.38	< 0.05	
	NT10_mean7	0.052	0.0087	5.96	< 0.0001	
	NEEDLE	-0.031	0.0100	-3.09	< 0.01	
	TIME	-0.005	0.0005	-9.73	< 0.0001	

Table 3.4. Definitions of terms used in mixed effects models for the *D. septosporum* red banding analysis.

Term	Definition
BANDS	Red bands per needle
NT7_min4	Number of days out of the last 4 in which the nightly minimum temperature (°C) has been ≥ 7°C
NT10_mean7	Number of days out of the last 7 in which the nightly mean temperature (°C) has been ≥ 10°C
NT18_max4	Number of days out of the last 4 in which the nightly maximum temperature (°C) has been ≥ 18°C
NT18_max7	Number of days out of the last 7 in which the nightly maximum temperature (°C) has been ≥ 18°C
DRH70_min4	Number of days out of the last 4 in which the daily minimum relative humidity (%) has been ≥ 70%
DRH70_min7	Number of days out of the last 7 in which the daily minimum relative humidity (%) has been ≥ 90%
DW40_mean4	Number of days out of the last 4 in which the daily mean leaf wetness (%) has been ≥ 90%
DW40_mean7	Number of days out of the last 7 in which the daily mean leaf wetness (%) has been ≥ 90%
NEEDLE	Sample size
TIME	Time as measured in Julian days

Table 3.5. Paired two-sample independent *t*-tests comparing weather variables between periods when *D. septosporum* signs were and were not observed.

Mean daily weather variable	Fruiting body development		Spore production	
	t_{4894}	p-value	t_{5560}	p-value
Temperature (°C)	8.6	$< 2.2 \times 10^{-16}$	7.61	3.193×10^{-14}
Relative humidity (%)	6.28	3.587×10^{-10}	5.74	1.025×10^{-8}
Leaf wetness (%)	-3.41	6.585×10^{-4}	-3.16	1.610×10^{-3}

Table 3.6. Mixed effect models for *D. septosporum* fruiting body development.

Model	Variable	Coefficient	SE	Test statistic	p-value	AIC
4 day temperature and relative humidity				t_{582}	< 0.0001	705
	Intercept	0.418	0.2557	1.63	0.1026	
	NT10_mean4	0.100	0.0210	4.77	< 0.0001	
	NEEDLE	-0.060	0.0145	-4.12	< 0.0001	
	TIME	0.002	0.0006	3.38	< 0.001	
4 day temperature and leaf wetness				t_{297}	< 0.0001	409
	Intercept	0.252	0.2188	1.15	0.2512	
	DT7_mean4	0.232	0.0535	4.33	< 0.0001	
	DW30_mean4	0.145	0.0473	3.05	< 0.005	
	DW90_mean4	-0.609	0.1133	-5.38	< 0.0001	
7 day temperature and relative humidity				t_{562}	< 0.0001	676
	Intercept	0.293	0.2586	1.13	0.258	
	DT6_min7	0.061	0.0140	4.37	< 0.0001	
	DRH90_max7	0.098	0.0295	3.30	0.001	
	NEEDLE	-0.069	0.0130	-5.30	< 0.0001	
7 day temperature and leaf wetness				t_{297}	< 0.0001	415
	Intercept	0.097	0.1859	0.52	0.6038	
	DT7_mean7	0.095	0.0269	3.53	< 0.001	
	DW30_mean7	0.105	0.0231	4.55	< 0.0001	

Table 3.7. Definitions of terms used in mixed effects models for the *D. septosporum* fruiting body analysis.

Term	Definition
BODS	Fruiting bodies per needle
DT6_min7	Number of days out of the last 7 in which the daily minimum temperature (°C) has been ≥ 7°C
DT7_mean4	Number of days out of the last 4 in which the daily mean temperature (°C) has been ≥ 7°C
DT7_mean7	Number of days out of the last 7 in which the daily mean temperature (°C) has been ≥ 7°C
NT10_mean4	Number of days out of the last 4 in which the nightly mean temperature (°C) has been ≥ 10°C
DRH90_max7	Number of days out of the last 7 in which the daily maximum relative humidity (%) has been ≥ 90%
DW30_mean4	Number of days out of the last 4 in which the daily leaf wetness (%) has been ≥ 30%
DW30_mean7	Number of days out of the last 7 in which the daily leaf wetness (%) has been ≥ 30%
DW90_mean4	Number of days out of the last 4 in which the daily leaf wetness (%) has been ≥ 90%
NEEDLE	Sample size
TIME	Time as measured in Julian days

Table 3.8. Site characteristics of lodgepole pine openings in the 2006 aerial survey conducted to assess the severity of the Dothistroma needle blight.

Timber Supply Area	Elevation (m)	Slope (%)	Distance from nearest water body (m)
Bulkley	711.72 ± 252.62	12.00 ± 8.07	189.14 ± 125.24
Cranberry	426.43 ± 137.44	14.19 ± 9.11	131.30 ± 95.93
Kalum	185.42 ± 137.53	10.73 ± 12.94	258.03 ± 199.51
Kispiox	543.35 ± 165.24	14.62 ± 11.03	240.58 ± 185.48
Nass	316.09 ± 150.16	11.13 ± 8.12	121.32 ± 116.29
Total	491.15 ± 230.32	13.29 ± 10.37	206.79 ± 170.37

Table 3.9. Logistic regression models for topographical effects on *D. septosporum* development.

Model	Variable	Coefficient	SE	Test statistic	p-value	AIC
y_5				z_{602}		258.4
	Intercept	-266	50.3	-5.28	< 0.0001	
	X	4.49×10^{-5}	8.75×10^{-6}	5.13	< 0.0001	
	Y	3.96×10^{-5}	7.65×10^{-6}	5.18	< 0.0001	
	Elev	-0.0132	2.26×10^{-3}	-5.85	< 0.0001	
	NE	1.42	0.444	3.21	< 0.01	
	W	0.991	0.423	2.34	< 0.05	
y_{10}				z_{602}		349
	Intercept	-250	39.2	-6.37	< 0.0001	
	X	5.05×10^{-5}	7.32×10^{-6}	6.90	< 0.0001	
	Y	3.67×10^{-5}	5.96×10^{-6}	6.17	< 0.0001	
	Elev	-0.0144	1.92×10^{-3}	-7.53	< 0.0001	
	NE	0.828	0.405	2.05	< 0.05	
	W	0.949	0.355	2.67	< 0.01	
y_{20}				z_{604}		449.3
	Intercept	-216	30.3	-7.11	< 0.0001	
	X	5.25×10^{-5}	6.16×10^{-6}	8.53	< 0.0001	
	Y	3.11×10^{-5}	4.59×10^{-6}	6.77	< 0.0001	
	Elev	-0.0137	1.51×10^{-3}	-9.04	< 0.0001	
y_{50}				z_{604}		681.7
	Intercept	-110	17.7	-6.25	< 0.0001	
	X	3.40×10^{-5}	4.32×10^{-6}	7.87	< 0.0001	
	Y	1.56×10^{-5}	2.67×10^{-6}	5.83	< 0.0001	
	Elev	-7.24×10^{-3}	7.21×10^{-4}	-10.03	< 0.0001	

Table 3.10. Definitions of terms used in logistic regression models for the *D. septosporum* topographic analysis.

Term	Definition
X	Eastings (west to east direction)
Y	Northings (south to north direction)
Elev	Elevation (m)
NE	Northeast-facing aspect
W	West-facing aspect

CHAPTER 4: DISCUSSION

4.1 Field observations of *D. septosporum* symptom development

Red bands and fruiting bodies were apparent on lodgepole pine (*Pinus contorta* var *latifolia* Dougl. Ex Loud.) needles at the start of the study period in June. Initial symptoms of *Dothistroma septosporum* (Dorog.) Morelet infection on pine needles are green translucent bands that become necrotic (Funk and Parker 1966; Peterson 1982). The majority of green translucent bands forming in the fall turn bright red and develop mature fruiting bodies the following spring (Funk and Parker 1966). During the establishment of study plots in May, low to moderate red banding was observed on selected sites as well as low fruiting body numbers. Red bands and fruiting bodies observed at the start of the study period are attributed to infections from the previous year, respectively indicating points of successful *D. septosporum* colonization and the amount of pathogen present.

In a lab-controlled environment, bright red bands form on pine needles from dothistromin injection within five days (Franich *et al.* 1986). Field observations of Austrian (*Pinus nigra* Arnold) and ponderosa (*Pinus ponderosa* Dougl. ex Laws.) found that the period in which red bands form on pine needles varies from four to seven weeks from the time that hyphae were detected entering stomata (Peterson and Walla 1978). Fruiting bodies appear within another three to seven weeks (Peterson and Walla 1978). In controlled inoculation studies on radiata pine (*Pinus radiata* D. Don) under various temperature and moisture regimes, conidia-bearing stromata appeared two to seven weeks after inoculation (Gadgil 1974). Excluding initial observations of red banding and fruiting bodies occurring together, the period in which fruiting bodies followed the appearance of red banding ranged

from ten days to seven weeks. This is consistent with both previous lab-based and field observations.

No red banding or fruiting bodies were observed on selected needles after late September, indicating that in the 2007 field season early fall weather conditions were no longer conducive for *D. septosporum* development. Though the absence of red banding in *D. septosporum* infected foliage has been observed in the field (personal observation and Alex Woods[1], personal communication, January 16, 2007), in this study the development of *D. septosporum* fruiting bodies was consistently observed on needles that had previously displayed *D. septosporum* red banding and fruiting bodies. The absence of red in bands was observed only when adjacent needle tissue was already dead. Bands were no longer counted when their color became indistinguishable from dead needle tissue. This timing varied widely among nodes and plots.

The red color of the bands is caused by the mycotoxin dothistromin, which is unclear in its role in pathogenicity (Bradshaw 2004). Dothistromin is known to rapidly degrade in needle tissue (Franich *et al.* 1986). However in the field dothistromin-containing red bands persist weeks after infection (Peterson and Walla 1978; Schwelm *et al.* 2009). Ongoing dothistromin production may defend establishment in needle tissue either from plant defense responses or competition from other needle decay fungi (Bradshaw 2006). Competition may increase as needle death progresses and secondary needle decay fungi begin to invade. As many as 27 fungal species have been isolated from needle tissue adjacent to red bands on *P. nigra* (Karadžić 1989). In lab-based pathogenicity tests mutant strains of *D. septosporum* unable to produce dothistromin are able to colonize, trigger lesion formation, and sporulate

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as well as dothistromin-producing wild-type isolates (Schwelm *et al.* 2009). From this evidence Schwelm *et al.* (2009) suggests that the main targets of dothistromin are needle-dwelling endophytes or latent pathogens, thus providing evidence contrary to the assumption that dothistromin is important in pathogenicity. Dothistromin production may continue until either resources are exhausted within the needle tissue or weather conditions are no longer conducive to growth, at which point the exclusion of secondary needle decay fungi is too expensive to maintain.

This competitive strategy is also consistent with the observed early summer peak in red banding development that gradually declined as spore production and fruiting body development peaked, reflecting a possible transition in resource utilization from competitive exclusion to reproduction as the summer progresses. This transition between dothistromin production and fruiting body development may be associated with a change in relative humidity, as this was the sole weather variable in which the observed daily means for red banding and fruiting body development were found to differ significantly. In the mixed effects models fruiting body development required $\geq 90\%$ maximum daily relative humidity levels, whereas red banding required a minimum relative humidity of $\geq 70\%$.

Red banding has been considered an unreliable diagnostic feature for *D. septosporum*, as it is not always brick red in color and can be confused with the symptoms of other needle diseases (Ivory 1994; Brown *et al.* 2003). It is also possible that in what is known to be a genetically diverse population in northwestern British Columbia (BC), variations of *D. septosporum* that are either dothistromin-deficient or producing only faint amounts of dothistromin are present. However, none were observed in this field study.

The occurrence of *D. septosporum* fruiting bodies peaked on lodgepole pine needles from July to August, gradually decreasing until late September. They generally persisted longer than the red banding from which they erupted. From the start of the study period *D. septosporum* fruiting bodies were easy to identify on the basis of a black fruiting body emerging from beneath a flap of needle epidermis. However fruiting bodies were no longer counted when they lost these identifiable characteristics or were indistinguishable from other emerging needle decay fungi. Other needle decay fungi observed on selected needles included *Davisomycella ampla* (J. J. Davis) Darker, *Hendersonia pinicola* (Wehm.), and *Lophodermium sp.* When diseased needles senesced or died these other fungal pathogens often developed, disrupting the appearance of *D. septosporum* red bands and fruiting bodies.

An unknown proportion of fruiting bodies observed in the fall were likely the result of secondary infections (infections resulting from spores released during the same season). However, it is not possible to calculate this proportion because the exact inoculation date of each needle cannot be determined, and due to overlap between primary and secondary infections. Peterson (1969; 1973) did not observe spores on *D. septosporum* fruiting bodies that appeared in the fall, noting that extensive infection can occur late in the summer and most stromata mature during the spring of the following year. This observation is consistent with our finding of no spore production after late August in spite of the highest observed frequencies of fruiting bodies during this time. Spore production peaked mid summer as red banding began to decline and prior to the late summer peak in fruiting body development. Fruiting bodies continued to emerge up to seven weeks after spores were no longer detected.

4.2 Weather conditions driving *D. septosporum* symptom development

Moist conditions appear to be the weather factors most closely correlated to the development of *D. septosporum* red banding and fruiting bodies. Red bands were observed on days with high average relative humidity (85.6 ± 8.4%) and were never observed when relative humidity was less than 57.8%. Fruiting bodies were also observed at plots with high average relative humidity (87.1 ± 7.2%) and never observed at plots with relative humidity levels less than 69.1%. High average leaf wetness also characterized plots at which red bands and fruiting bodies were observed (55.4 ± 15.2% and 57.0 ± 14.4% respectively). Though humidity and leaf wetness levels were consistent with lab-based findings for optimum *D. septosporum* development conditions, optimal temperatures in the field were lower. Temperatures ranging from 15-20°C, when accompanied by extended periods of moisture, are optimal for *D. septosporum* development in lab-based studies (Ivory 1972; Gadgil 1974; Bulman *et al.* 2004). In this study moderate temperatures were sufficient for red banding (12.8 ± 1.9°C) and fruiting body (12.9 ± 1.9°C) development. Neither symptoms were observed on days where the average daily temperature was less than 8°C. This disparity between lab and field observations was also observed in a field study for *Phytophthora ramorum* on oak (*Quercus sp.*) and tanoak (*Lithocarpus densiflorus* (Hook & Arn.) Rehd.) (Condeso and Meentemeyer 2007). High disease severity was also associated with lower temperatures in the field than the laboratory-determined optimal range for pathogen reproduction (Condeso and Meentemeyer 2007).

Temperature thresholds for *D. septosporum* development were consistent with observations from other field studies. Four- and seven-day temperature and leaf wetness models found that mean daily temperatures above 7°C significantly influenced the

development of *D. septosporum* fruiting bodies. Temperature and relative humidity models were less consistent. Only mean nightly temperature above 10°C was significant in the four-day model, whereas in the seven-day model daily minimum temperature above 6°C was significant. In Serbia *D. septosporum* infections did not occur on *P. nigra* in months when the average temperature was less than 7°C (Karadžić 1989). In a 3-year controlled field study in New Zealand involving two weeks of exposure of individual seedlings to infection, Gilmour (1981) found that temperature thresholds for *D. septosporum* disease symptoms varied year to year, ranging from 7°C to 12°C. Although Gilmour (1981) could not demonstrate a correlation between infection levels and temperature or leaf wetness, length of the pre-reproduction period was significantly negatively correlated with mean air temperature. That is, stromata appeared sooner after infection with warmer temperatures.

In all models for red banding development nightly temperatures were the most significant variables. In four-day models, the number of days in which nightly minimum temperatures reached 7°C and maximum temperatures reached 18°C were found to significantly influence the development of *D. septosporum* red bands. In seven-day models, red banding was significantly influenced by the number of days in which nightly mean temperatures reached 10°C and maximum temperatures reached 18°C. Whether degradation of dothistromin is plant-induced or photolytic remains unclear (Bradshaw and Zhang 2006). It is known that dothistromin is broken down more efficiently in light (80%) compared to dark (5-10%) (Franich *et al.* 1986). It is possible that *D. septosporum* colonization may be affected by on night temperatures, when it is warm enough for the fungus to be active in needle tissue while avoiding the photolytic degradation of dothistromin in order to maintain competitive exclusion.

High relative humidity was significant in explaining the development of *D. septosporum* symptoms. Daily minimum relative humidity levels above 70% were significant in both four- and seven-day temperature and relative humidity models for *D. septosporum* red banding. Ninety percent daily maximum relative humidity was significant in the seven-day temperature and relative humidity model for *D. septosporum* fruiting body development. No relative humidity variables were retained in the four-day model. Most studies examining the role of relative humidity have focused on the germination of conidia in a lab setting. Sheridan and Yen (1970) found conidia germinated well at 98-100% relative humidity, and some conidia appeared to germinate at relative humidities as low as 76%. Our findings are consistent with the suggestion that symptom development at lower temperature is dependent on extended periods of high humidity (Gilmour and Crockett 1972, cited in Bradshaw 2004).

In all models where relative humidity was tested against leaf wetness, only leaf wetness variables were retained. For *D. septosporum* red banding minimum daily leaf wetness above 40% was significant in both four- and seven-day temperature and leaf wetness models. For *D. septosporum* fruiting body development, daily mean leaf wetness above 30% was significant in the four- and seven-day models. Based on field observations, 30-40% leaf wetness is the threshold at which the surfaces of pine needles are wet. The presence of moisture for red banding and fruiting body development supports the emphasis of leaf wetness in *D. septosporum* development, particularly when foliage is kept continuously wet (Gadgil 1974; Gadgil 1977). In lab settings stromata develop sooner with longer wetness periods (Gadgil 1974), and in the field no infection is observed when leaf wetness periods are less than ten hours (Gilmour 1981). A negative correlation was found for 90% daily mean leaf wetness and fruiting body development in the four-day model, suggesting that the role of

leaf wetness has an upper limit. Days in which the mean leaf wetness would have been higher than 90% would be days with frequent rain and slow drying conditions, possibly associated with cooler temperatures. Although wet conditions encourage *D. septosporum* development, conditions in which needles are soaking for extended periods of time may discourage the disease by limiting respiration.

In all red banding models the number of observed red bands decreased with increasing sample size and as time progressed. We observed *D. septosporum* red banding to lose its distinct color and become indistinguishable from dead adjacent needle tissue, accounting for their loss over time. A positive coefficient for TIME was observed in one fruiting body model that is consistent with the observed late summer peak in fruiting body development. Two fruiting body models also had negative coefficients for NEEDLE. The increase in observed symptoms with decreasing sample size can be explained by considering the general health of selected nodes. Nodes retaining the maximum sample of ten marked needles were more likely to be those with less disease. Healthy green needles were more easily retained, whereas infected needles tended to drop as they became progressively diseased. Nodes that lost needles were generally heavily infected, bearing few to no healthy green needles. This would have resulted in an apparent increase in disease severity with decreasing sample size.

4.3 Spore production and frequency of the sexual stage

Conidia were detected from late June to late August in 2007. The average temperature, relative humidity, and leaf wetness on days which conidia were detected were 13.3 ± 1.8°C, 85.7 ± 5.3%, and 58.3 ± 12.4% respectively. Unfortunately spore production data were insufficient for statistical analyses. The spore sampling procedure was chosen as an

alternative to using spore traps as previous work with volumetric spore traps yielded poor results due to equipment failure (Lewis, unpublished data). In future studies the use of petroleum-coated slides or non-volumetric traps may be a better alternative for capturing *D. septosporum* conidia, as has been demonstrated by Peterson (1973).

High levels of genetic diversity have been observed in the *D. septosporum* population in northwestern BC (Dale 2008). In this study ascospores were never detected in the spore sampling procedure. They were detected in ascomata during the fruiting body dissections, confirming the presence of the sexual stage in 0.00075% fruiting body dissections. This suggests that sexual reproduction is rare relative to asexual reproduction.

The rarity of the sexual stage was expected even though genetic variability of the *D. septosporum* population in northwestern BC is known to be high (Dale 2008). Through sectioning and observing 200 stromata from naturally infected needles of *P. contorta* on southern Vancouver Island, the sexual stage of Dothistroma was observed only 4-5% of the time (Funk 1979). The sexual stage was never observed in a field study by Peterson (1973), who was monitoring *P. nigra* and *P. ponderosa* for Dothistroma development and spore production in Nebraska, USA. This provides evidence that the primary dispersal of *Mycosphaerella pini* E. Rostrup is through the asexual conidia, as only small amounts of sexual recombination are required to have a large impact on population structure (Dale 2008).

There are several reasons why the sexual stage may have been under-estimated in this study. The presence of ascospores was part of the identification criteria for ascomata. This was consistent with the use of conidia to confirm the identity of stromata, which were detected on the majority of stromata dissected. However this requirement may have resulted

in ascomata numbers being under-estimated because the presence of immature ascomata was not recorded and due to the similar appearance of both reproductive stages, immature sexual fruiting bodies were counted as asexual. It is possible that sampling once a week was not sensitive enough to capture the period in which sexual reproduction occurs, though this would be unlikely if ascospore production lasts about a month (Funk and Parker 1966). In southern BC Funk and Parker (1966) reported that conidia are produced from early spring to late summer or early fall, while ascospores are produced in early June. In northwestern BC if sexual reproduction had occurred primarily in late spring, overlapping only briefly with conidia production, it may have been missed. The sexual stage may also be more of a response to stressful environmental conditions, which could explain why ascomata were detected in mid-August and late-September samples when environmental conditions would have started to decline. Finally, it may be that the sexual stage develops primarily in needle tissue killed as a result of infection. In Serbia, ascomata usually formed when needles were completely necrotic, most often on 2-3 year old needles of Austrian pine (Karadžić 1994). If this is the case, then selecting Dothistroma-infected needles older than one-year might have more accurately assessed the frequency of the sexual stage.

4.4 Influence of topography on *D. septosporum* development

Northings, eastings, and elevation had significant influence on the disease severity of pine-leading plantations at all four functional live crown (FLC) thresholds. Stands unlikely to recover were more frequently encountered in the north and east directions of the study area. At 5% and 10% FLC thresholds aspect also had a significant influence. Slope and proximity to nearby water bodies were not found to have any effect on disease severity.

Disease severity increased with decreasing elevation at all FLC thresholds. This effect can be explained by both temperature and humidity requirements for the disease. Areas of higher elevation have lower average temperatures than areas of lower elevation (Kimmins 1997). Cool air at higher elevation also holds less water, resulting in a cooler, drier environment less conducive to *D. septosporum* disease development. This finding is consistent with the results of a pilot study examining the effects of site characteristics on *D. septosporum* disease severity, in which elevation was the only significant factor explaining the severity of *D. septosporum* infection (Braun, unpublished data). It is also consistent with observations from a growing Dothistroma needle blight outbreak on *Pinus pallasiana* D. Don in southern Ukraine, where the disease is rarely observed on trees growing at low densities on wind-exposed slopes at higher elevations (Barnes *et al.* 2008). This finding supports the suggestion by Marks and Hepworth (1986) that 'hot spots' for *D. septosporum* infection are in areas where cool air can pool, such as in low-lying areas.

At 5% and 10% FLC thresholds, west- and northeast-facing pine-leading stands experienced higher disease severity. This suggests that at FLC thresholds lower than 20% the fine-scale differences in aspect may determine the rate at which trees advance from growth cessation to death. Warmer, drier microclimates are experienced on south-facing slopes whereas cool, moist microclimates generally characterize northeast aspects and lower slopes (Spurr and Barnes 1980). *D. septosporum* symptom development at lower temperatures is dependent on extended periods of high humidity (Gilmour and Crockett 1972, cited in Bradshaw 2004). Weather analysis results in this study support the emphasis of leaf wetness in *D. septosporum* development, particularly when foliage is kept continuously wet (Gadgil

1974; Gadgil 1977). In spite of warmer temperatures, the drier microclimate of south-facing slopes appears to discourage *D. septosporum* development.

Proximity to the nearest river or lake was not related to disease severity. While the high humidity requirements for *D. septosporum* infection and reproduction are met by the presence of fog and mist, high severity areas appear to be places where fog is not necessarily generated but retained. However it is possible that the lack of correlation with rivers is a reflection of how far the disease has progressed. Earlier sampling might have revealed original outbreak epicenters. It is also possible that there was too much sampling near rivers and lakes (mean distance of 206.8 ± 170.4 m) to reveal measurable differences.

Though Dothistroma infection and reproduction was not influenced by nearby water bodies, the negative correlation with increasing elevation suggests that disease severity may not be so much a result of readily available fog to generate moisture on needle surfaces, but the result of pooling cool air in low-lying areas. In the case of *Gremmeniella abietina* (Lagerb.) Morelet infection in Scots pine (*Pinus sylvestris* L.), the relative elevation of the site, in relation to the nearest river or lake, was more important than the absolute site elevation, resulting in a negative correlation between mortality and local elevation (Nevalainen 2002). Similar to *D. septosporum*, the most severely damaged stands of Scots pine are found in river valleys (Sairanen 1990). It is possible that a more in-depth analysis might have revealed a stronger relationship between rivers and disease severity.

The potential influence of topographic variables from the proposed Dothistroma Needle Blight Hazard Rating Approach were illustrated in thematic maps generated from logistic regression models using the 10% and 20% FLC thresholds. Both models produced similar graphic results, displaying road and river corridors as high risk areas for severe

Dothistroma infection in the Cranberry Timber Supply Area (TSA). The 10% FLC logistic regression prediction map may be more sensitive as it incorporates aspect into its predictions. Accuracy could be improved by generating double precision coverages (64-bit floating point) as opposed to single precision coverages (32-bit floating point). This would require working with datasets twice as large, which would involve increased processing time. Accuracy could also be improved by generating finer resolution point features from which to generate northings and eastings rasters. Map scale could also be improved through using Geogratis 1:50,000 or BC TRIM 1:20,000 grids to instead of 1:250,000 DEMs to digitize the logistic regression models. Issues of scale would require further attention in validating the map output. Although these prediction maps could not be validated within the scope of this study, the precision of these predictive models could be verified through iterative testing or by comparison with Permanent Sample Plot data from the Prince George Forest District where *D. septosporum* symptoms have been observed (personal observation and Kathy Lewis[1], personal communication, May 16, 2006). Future studies could also focus on incorporating weather variables into the models as well as generating prediction maps for the larger TSAs involved in this study.

4.5 Conclusions

In northwestern BC *D. septosporum* symptoms can be observed on lodgepole pine from early June until late September. In this study red banding was consistently observed to precede and accompany the appearance of *D. septosporum* fruiting bodies. The red color of *D. septosporum* bands fades over time and should not be used as a conclusive diagnostic feature, particularly in late summer. With moderate temperatures, high humidity, and wet

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needles *D. septosporum* can develop on lodgepole pine needles. Optimal temperatures observed in the field are lower than those identified in lab-based studies, indicating that *D. septosporum* symptoms will develop at moderate temperatures if humidity and leaf wetness levels are sufficient.

Although *D. septosporum* conidia were detected from late July until late August, the spore trapping procedure used in this study may not accurately reflect the timing of spore production. Ascospores were never detected by means of the needle dip method, and were detected on ascomata during fruiting body dissections. Ascomata were identified only twice from 2670 fruiting body dissections, suggesting that sexual reproduction is infrequent in the *D. septosporum* population of northwestern BC.

Elevation is the most consistent topographic characteristic influencing *D. septosporum* severity, which is greater at low elevations where microclimatic conditions are the most favourable for disease development. Aspect was the second most consistent characteristic; south-facing aspects discouraged *D. septosporum* severity due to their drier microclimates. Slope and proximity to rivers and lakes had no influence on *D. septosporum* severity. A disease hazard rating approach was proposed based on these topographic variables, and logistic regression models were projected as probability maps to illustrate graphic-generating use of these results. From these results it can be concluded that when sufficient inoculum levels are present, low-lying areas where moist air can be retained for long periods facilitate *D. septosporum* development.

4.6 Literature Cited

Barnes, I., Kirisits, T., Akulov, A., Chhetri, D. B., Wingfield, B. D., Bulgakov, T. S., and Wingfield, M. J. 2008. New host and country records of the Dothistroma needle blight pathogens from Europe and Asia. Forest Pathology 38(3):178-195.

Bulman, L. S., Gadgil, P. D., Kershaw, D. J., and Ray, J. W. 2004. Assessment and control of Dothistroma needle-blight. New Zealand Forest Health Research Collaborative, Forest Research Bulletin 229.

Bradshaw, R. 2006. Molecular Techniques: What can they tell us about Dothistroma needle blight? In: Jackson, M. B. comp. 2007. Proceedings of the 54th Annual Western International Forest Disease Work Conference; 2006 October 2-6; Smithers, BC. Missoula, MT: US Department of Agriculture, Forest Service, Forest Health Protection. p 93-95.

Bradshaw, R. E. 2004. Dothistroma (red-band) needle blight of pines and the dothistromin toxin: a review. Forest Pathology 34(3):163-185.

Bradshaw, R. E., and Zhang, S. 2006. Biosynthesis of dothistromin. Mycopathologia 162:201-213.

Brown, A., Rose, D., and Webber, J. 2003. Red band needle blight of pine. Forestry Commission [Information Note] 49:1-6.

Condeso, T. E., and Meentemeyer, R. K. 2007. Effects of landscape heterogeneity on the emerging forest disease sudden oak death. Journal of Ecology 95:364-375.

Dale, A. 2008. Dothistromin production and its relationship to genetic diversity in the fungal pathogen *Dothistroma septosporum*. M.S. thesis. University of Northern British Columbia, Prince George.

Franich, R. A., Carson, M. J., and Carson, S. D. 1986. Synthesis and accumulation of benzoic acid in Pinus radiata needles in response to tissue injury by dothistromin, and correlation with resistance of *P. radiata* families to *Dothistroma pini*. Physiological and Molecular Plant Pathology 28:267-286.

Funk, A. 1979. Sexuality in *Scirrhia pini*. Canadian Forestry Service Bi-monthly Research Notes 35:14.

Funk, A., and Parker, A. K. 1966. *Scirrhia pini* n. sp., the perfect state of *Dothistroma pini* Hulbary. Canadian Journal of Botany 44:1171-1176.

Gadgil, P. D. 1977. Duration of leaf wetness periods and infection of *Pinus radiata* by *Dothistroma pini*. New Zealand Journal of Forestry 7(1):83-90.

Gadgil, P. D. 1974. Effect of temperature and leaf wetness period on infection of *Pinus radiata* by *Dothistroma pini*. New Zealand Journal of Forestry Science 4:495-501.

Gilmour, J. W. 1981. The effect of season on infection of *Pinus radiata* by *Dothistroma pini*. European Journal of Forest Pathology 11:265-269.

Gilmour, J.W., and Crockett, F. 1972. *Dothistroma pini* project: monitoring of infection patterns in the field. Forest Research Institute Report No. 53. New Zealand Forest Service.

Ivory, M. H. 1994. Records of foliage pathogens of *Pinus* species in tropical countries. Plant Pathology 43:511-518.

Ivory, M. H. 1972. Infection of *Pinus radiata* foliage by *Scirrhia pini*. Transactions of the British Mycological Society 59(3):365-375.

Karadžić, D. M. 1994. Picea omorika - a new host of *Dothistroma septospora*. European Journal of Forest Pathology 24:300-303.

Karadžić, D. 1989. Scirrhia pini Funk et Parker. Life cycle of the fungus in plantations of *Pinus nigra* Arn. In Serbia. European Journal of Forest Pathology 19:231-236.

Kimmins, J. P. 1997. Forest Ecology: A Foundation for Sustainable Management. Prentice-Hall Inc. 2nd ed. Upper Saddle River, New Jersey, USA.

Marks, G. C., and Hepworth, G. 1986. Effect of Certain Features of a Stand of *Pinus radiata* on *Dothistroma septospora* Needle Blight. Australian Forest Research 16:223-229.

Nevalainen, S. 2002. The incidence of *Gremmeniella abietina* in relation to topography in southern Finland. Silva Fennica 36(2):459-473.

Peterson, G. W. 1982. Dothistroma Needle Blight of Pines. Available from: http://www.na.fs.fed.us/spfo/pubs/fidls/dothistroma/doth.htm Accessed Mar 3 2006.

Peterson, G. W. 1973. Infection of Austrian and Ponderosa Pines by *Dothistroma pini* in Eastern Nebraska. Phytopathology 63:1060-1063.

Peterson, G. 1969. Dothistroma needle blight of Austrian and ponderosa pines: epidemiology and control. Phytopathology 57:437-441.

Peterson, G. H., and Walla, J. A. 1978. Development of *Dothistroma pini* upon and within needles of Austrian and ponderosa pines in eastern Nebraska. Phytopathology 68:1422-1430.

Sairanen, A. 1990. Site characteristics of Scots pine stands infected by *Gremmeniella abietina* in Central Finland, I: Mineral soil sites. Acta Forestalia Fennica 216:27.

Schwelm, A., Barron, N. J., Baker, J., Dick, M., Long, P.G., Zhang, S., and Bradshaw, R.E. 2009. Dothistromin toxin is not required for dothistroma needle blight in *Pinus radiata.* Plant Pathology 58:293-304.

Sheridan, J. E., and Yen, C. C. 1970. A note on the effect of temperature and relative humidity on the germination of conidia of a New Zealand isolate of *Dothistroma pini* Hulbary. New Zealand Journal of Botany 8:658-60.

Spurr, S. H., and Barnes, B. V. 1980. Forest Ecology. Krieger Publishing Company. 3rd ed. Malabar, Florida.